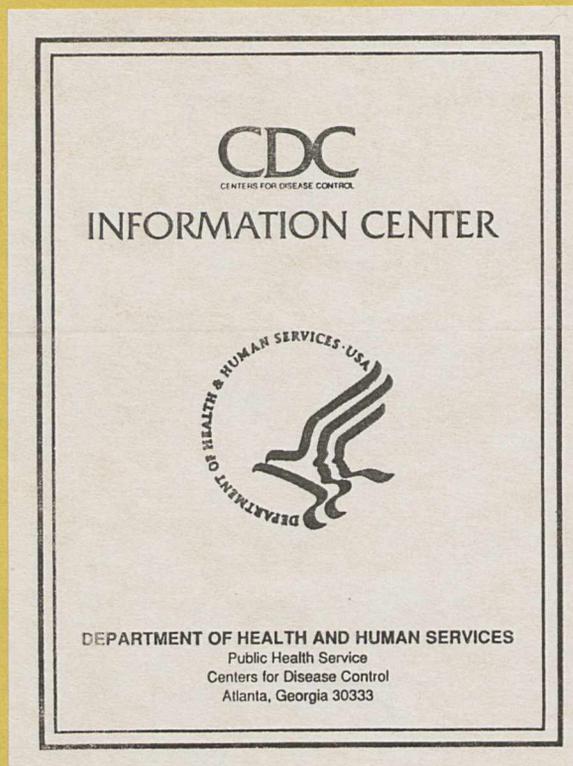


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Air Filtration of Microbial Particles

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I. Introduction

Summary

Air filtration is an efficient means of removing microbial particles as small as one micron from air. Various types of filters have been evaluated, using as the test simulant bacterial organisms aerosolized in particles with diameters of 1 to 5 microns. Based on results of these tests, filters have been placed into four categories: roughing (10 to 60 percent efficient), medium-efficiency (60 to 90 percent efficient), high-efficiency (90 to 99 percent efficient), and ultra-high-efficiency (99.99+ percent efficient).

To remove organisms from the air at the lowest cost, a filter system is recommended. Such a system generally consists of low-efficiency filters placed upstream from higher-efficiency units. The upstream filters protect the high-efficiency filters from premature loading of dust and consequent frequent and expensive replacements. Removal efficiency of an entire filter system can easily reach 95 percent, a level considered to be satisfactory for most situations, and can achieve 99.99+ percent when necessary. Other air cleaners, including washers, electrostatic precipitators, and ultraviolet devices, generally have not provided consistently high levels of removal efficiency under field conditions.

The efficiency of a filter in removing microbial particles should not be evaluated directly by the standard dust tests, such as that of the National Bureau of Standards. Air cleaning devices can be tested with accuracy in a test device in the laboratory using nebulized organisms, such as *Bacillus subtilis* var. *niger*, or in the field using naturally occurring organisms in the air. The test procedures can be carried out by any bacteriological laboratory with moderate resources.

Filters can be installed in most ventilation systems, but the higher the design efficiency, the more carefully the high efficiency filters must be sealed in their frames. Filters contaminated with pathogenic organisms can be decontaminated in place or immediately after removal by the use of formaldehyde, ethylene oxide, or heat. No residual germicide is known to be capable of reducing measurably the organisms trapped on filters except under conditions of high humidity.

I. Introduction

The authors of this monograph have been associated for several years with the problem of protecting personnel against disease-producing organisms found in hospitals or in biological laboratories. Although there are various means of cleaning air, the authors' experience has been widest in the use of air filtration as a method for removing bacteria from the air. This monograph is written for the specific purpose of making the benefits of this experience available to personnel such as architects, engineers, hospital administrators, and research investigators concerned with the control of pathogenic biological organisms. Although this report is oriented to biological air filtration, certainly the information also is applicable to industries using radioactive materials and to others, such as precision instrument industries or space-craft projects that require physically clean air. Other methods of air purification, such as electrostatic precipitation and air washing, that remove the particles from the atmosphere, or ultraviolet light and incineration, which destroy the biological organisms without removing them from the air, also have been used. However, they are only mentioned here as a means, and are not discussed extensively in this monograph.

In the past, filter manufacturers have been interested primarily in the reduction of airborne dust concentration, but only to a level that could be tolerated by the people inhaling the air, or to a level demanded by the industrial processes involved. Air cleanliness was considered satisfactory if dust particles were not visible and if there were no disagreeable effects such as irritation or odor. It was felt that any further purification would result in prohibitive filter cost, since

initial and operating costs rise as the efficiency of the filter increases.

In recent years, the subjects of microbiological air pollution, air sanitation, and air cleaning equipment have gained importance. Considerable information is now available concerning the immediate and latent ill effects caused by inhalation and retention of foreign airborne particles and bacteria.

Present knowledge indicates that those particles approximately 1 to 5 microns in diameter and those less than 0.2 micron in diameter are most effective for penetration and retention in the deep pulmonary spaces (1). However, larger particles bearing many organisms may be of importance in the infection of open wounds. Therefore, the authors, although fully aware of the difference in importance of various size particles, suggest that equal emphasis be given to the removal, or inactivation, of biological particles of all sizes from the air used in critical spaces.

The data on the performance of filters, filter media, and other cleaning devices designed for the removal of dust and bacteria from the air sometimes vary considerably from the performance claimed by manufacturers, usually because of differences in the numerous methods of evaluation and variation in test aerosols. Performance of filters to be discussed in this monograph is evaluated on the basis of biological test procedures developed by the authors. It should be pointed out that most filter evaluations published, to the present time, have been based on physical test methods. The results of these methods at times do not closely correlate with results of test methods using viable bacterial particles.

II. Methods of Air Cleaning

In general, most air cleaning equipment used to remove dust, mist, or fumes from air will also remove some bacteria. There are available many different types of air cleaning equipment. In most cases this equipment has been designed for special purposes, such as the removal of zinc fumes with particle sizes less than 0.1 micron or particles of chemical sprays such as acid mists (fig. 1). Physical methods involved in air cleaning include (a) gravitational, (b) inertial, (c) filtration, (d) washing, and (e) electrostatic precipitation. In general, most air cleaning equipment will remove from the air the percentages of bacteria in the 1- to 5-micron range of particle size shown in table 1. However, if the bacteria are associated with dust particles to give a particle size greater than 5 microns, the efficiency will be higher than indicated.

Filtration

When maximal removal of bacteria or radioactive particles from the air is required, filtration should be used. It is by far the most efficient and practical method of removing small particles from the air, particularly when an approach to sterility is required. The general principles of air filtration will be discussed briefly. For a more detailed discussion of the theory, the publications of La Mer *et al.* (2), Langmuir and Blodgett (3), Greene and Lane (4), and Rodebush *et al.* (5) are recommended.

Very few practical air filters depend upon screening or sieving action to remove the suspended material. Since the interstices of a screening-type filter must necessarily be smaller than the smallest particle to be removed, the resistance to air flow is high. As the surface becomes covered with collected material, resistance rises and ultimately air flow stops as all the interstices become plugged.

All practical aerosol filters consist of randomly oriented fibers of various materials

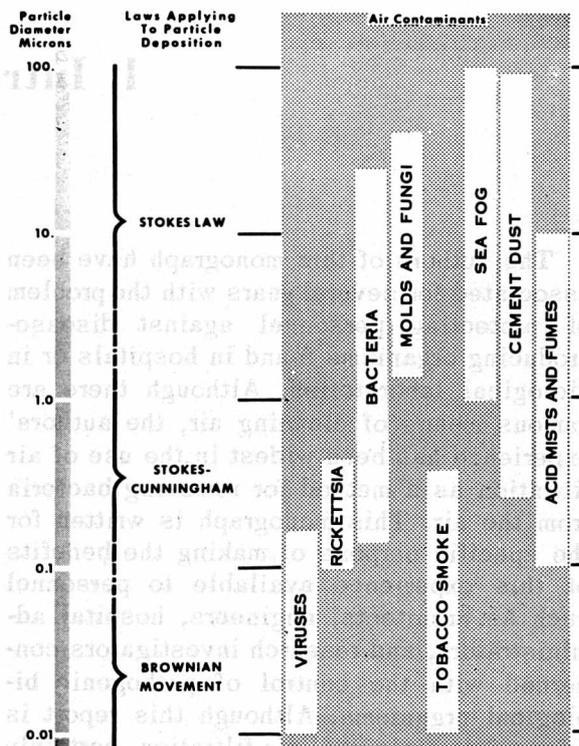


Figure 1. Size range of airborne particles.

placed in such a manner that most of the open spaces or interstices are much larger than the diameter of the particles to be removed. The filtering action depends upon the particles contacting and adhering to the fibers or collecting surface.

There are several mechanisms that may cause suspended particles to impact on the fibers. These may be by (a) direct interception, (b) deposition in accordance with Stokes' Law, (c) inertial effect, (d) diffusion, and (e) electrostatic effect. Direct interception and deposition have less effect in removing particles in filters than the latter three mechanisms.

The first mechanism, direct interception, is restricted to particles whose centers remain in a given streamline. It occurs if the particles are too large to show appreciable Brownian motion but are too small to be appreciably subject to Stokes' Law.

The second mechanism is settling according to Stokes' Law governing rate of fall. If a particle is large enough, it will not coincide with any streamline in the air flow, but will be deposited on the upper surfaces of the filter fibers. The rate of deposition will vary with particle size, concentration, and the total area that the upper surfaces of the fibers project into a horizontal plane. The rate of fall of particles less than 0.3 micron in diameter is so low that this mechanism is probably negligible in removing small particles in filters.

The third mechanism that will cause particles to collect on the filter fibers is the inertial effect. The forces of impaction are generally more effective for collection of particles one micron in diameter and larger. As air flows through a filter, it must continually change direction to permit flow around the randomly oriented filter fibers. As a result of inertial effects, those particles with sufficient mass continue in their original paths and strike the filter fiber despite the change in the path of the air flow. Other micro-aerodynamic forces may also be involved in this method of impaction, but nevertheless tests using bacterial organisms (1- to 5-micron diameters) have shown that impaction of particles on fibers is improved as the air velocity is increased

through the filter material (6). This is true because the inertial force is directly proportional to the square of the air velocity and inversely proportional to the radius of curvature of the air stream. It also has been found that decreasing the fiber diameter increases the collection efficiency (7).

The fourth mechanism, diffusion, pertains primarily to small-particulate aerosols. It accounts for the impaction of almost all particles having a diameter less than 0.3 micron. Fine particles of this magnitude diffuse in a manner similar to molecular diffusion, and in the case of passage through a filter are further subject to the laws governing isotropic turbulence which occurs when the eddying motion is randomly distributed (8). Contrary to the action with larger particles, a decrease in air velocity through the filter increases the deposition of small particles, since they remain within the filter configuration for a longer time. This results in greater opportunities for impaction by the diffusion process. Concomitantly, there is a decrease in deposition of large particles at low velocity because the inertial effect is much less. This is why most commonly used filters have greater collecting efficiency at higher velocities, particularly when the particles are large or in high concentrations.

TABLE 1. EFFICIENCY RANGE OF DEVICES FOR REMOVING BIOLOGICAL PARTICLES (1 TO 5 μ) FROM AIR

Cleaning Device	Bacterial Removal to be Expected (Percent)
Ultra-high-efficiency filters	99.99+
High-efficiency filters	90 to 99
Medium-efficiency filters	60 to 90
Roughing filters: fibrous, metallic, oiled, and screen types	10 to 60
Electrostatic precipitators	60 to 90
Air washers and scrubbers (low-pressure-drop type)	20 to 90

Still another process by which particles are deposited is by the electrostatic charge that may be present on the filters and particles when air at low humidity passes over the fibers. Certain types of filters may acquire both positive and negative electrostatic charges in various areas of the filter mass. These charges may be strong forces in the removal of particles from an air stream.

For the most efficient filter, it is desirable to have the fiber diameter less than the diameter of the smallest particles to be removed from the air. The use of small-diameter fibers increases greatly the deposition area of a filter and at the same time increases the free space, thereby reducing resistance to air flow. Small fibers require support to prevent their being packed together causing increased resistance. However, this is usually overcome by the use of a binder which holds the fibers in position. This allows the particles to deposit on the inner fibers of the filters, thereby providing greater loading capacity.

After particles are deposited they are held in place by electrostatic and adhesive forces between the particles and fibers. Coating fibers with an adhesive such as oil may increase retention. The ability of particles to remain on fibers is more dependent on the nature of the adhesive force than it is on fiber size or particle size until particle agglomerates become large enough that their cross sections produce air resistance sufficient for detachment.

Efficiency of all filters considered in this monograph is based on their efficacy in removing from the air bacteria 1 to 5 microns in diameter. Filters will be divided into four categories according to their efficiency and use. The terminology selected should not be considered as identical to that used by manufacturers of air cleaning equipment. These categories, which are given in table 1, are (a) roughing filters, (b) medium-efficiency filters, (c) high-efficiency filters, and (d) ultra-high-efficiency filters.

Roughing Filters

Roughing filters (fig. 2) are commonly

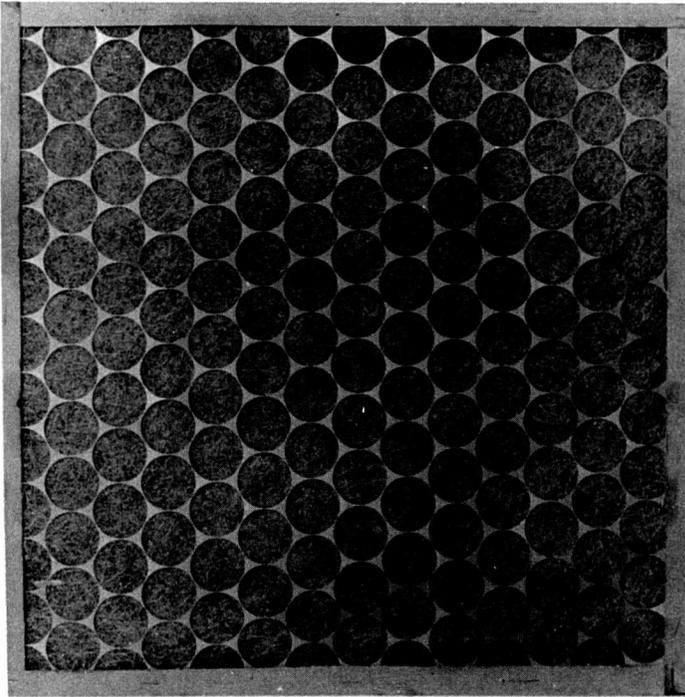
used when large amounts of contamination and debris are in the air. Roughing filters will remove the bulk of large airborne particles and some will remove 10 to 60 percent of the bacteria and other particles of a similar size (1- to 5-micron diameter); however, most remove less than 50 percent of 1- to 5-micron particles. Roughing filters may also be used as prefilters for higher efficiency filters to remove the "sticks and stones" and to reduce "loading" of the more expensive filters.

Two types of roughing filters in general use are the viscous-coated and the dry. Viscous filters are composed of woven metal screens or loosely packed fibers of animal hair, hemp, glass wool, or synthetics. The fibers are frequently coated with an adhesive substance, usually an oil, which aids in retaining the trapped particles. In some instances, these filters are constructed for indefinite use and can be cleaned and re-oiled when the fibers become loaded. A metal screen filter, which consists of a metal screen belt that moves across the air stream, is automatically cleaned by passing through an oil or water bath at the bottom of the filter unit, where the screen is cleaned and rewetted. The dust collects as sludge in the bath.

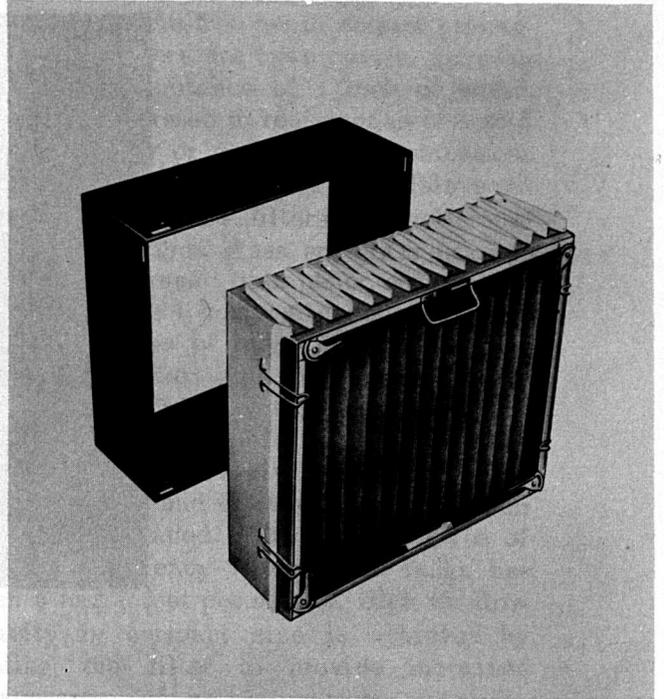
The dry type of roughing filter is composed of loosely packed glass or other fibers, cotton batting, or paper. In general, it offers more resistance to the passage of air (approximately 0.1 inch of water at rated air flows) and has a higher filtration efficiency than the viscous type. However, dry filters cannot be recleaned and must be discarded when the resistance to air flow becomes excessive. Filters used in commercial and small home unit air conditioners or furnaces are made of loosely packed hair or similar fiber or of woven metal in various forms. This type of filter removes only a small portion of airborne bacteria.

Medium-Efficiency Filters

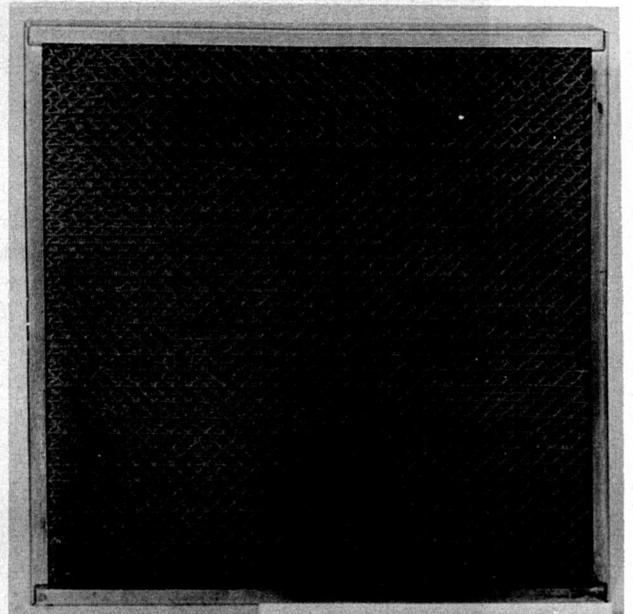
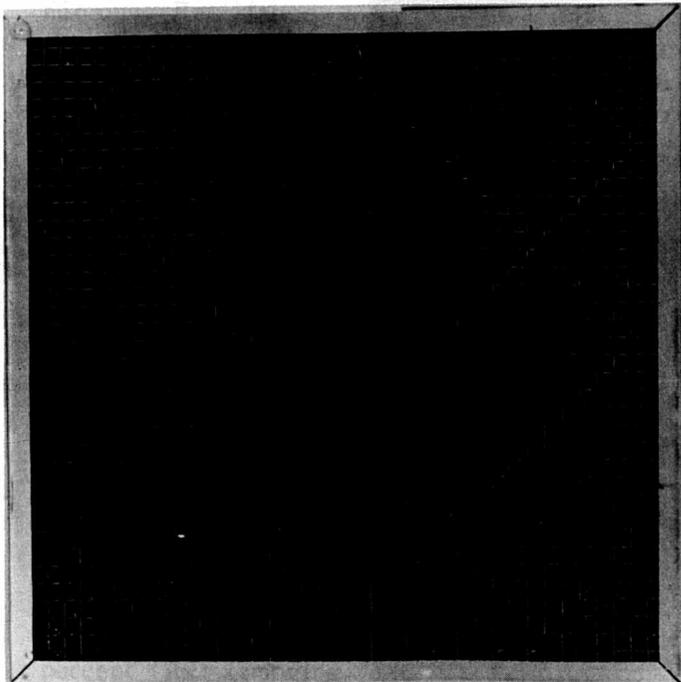
Medium-efficiency filters (fig. 3) remove 60 to 90 percent of the bacteria and other particles in the 1- to 5-micron diameter range. The filter material is usually com-



High-efficiency biological filters (fig. 4) remove 99 to 99.99 percent of all particles in the 1- to 3-micron diameter range. The filter media are chiefly glass fibers, good grades of filter paper, and asbestos fibers. The diameter of the fiber ranges from 1 to 3

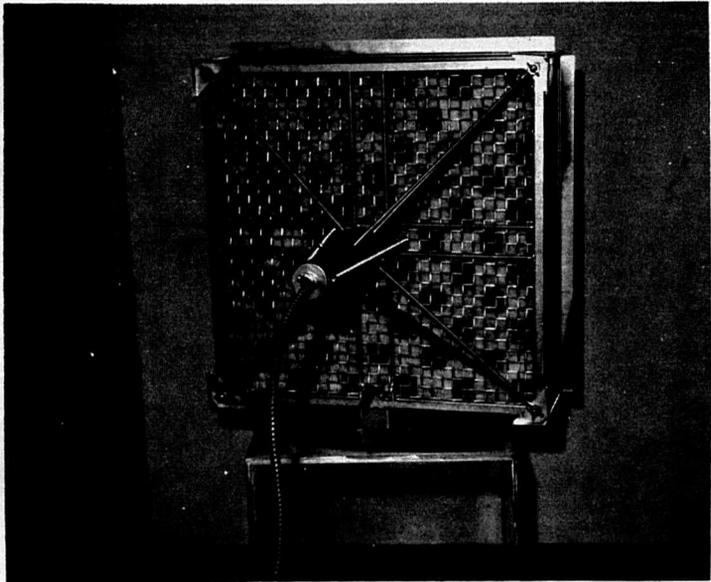


The ultra high-efficiency filter was originally developed by the U. S. Army Chemical Corps for use in gas masks and in building filtration systems for removing bacteria and other particles not removed by charcoal



having a diameter of 1 to 3 microns. Ultra high-efficiency filters are used by the pharmaceutical, electronic, and other industries for removing all particles down to

Figure 2. Roughing filters



...flow
 ...
 ...
 ...
 This allows the particles to deposit on the inner fibers of the filters, thereby providing greater loading capacity.

After particles are deposited they are held in place by electrostatic and adhesive forces between the particles and fibers,

...the fibers become loaded. A metal screen filter, which consists of a metal screen bolt that moves across the air stream, is automatically cleaned by passing through an oil or water bath at the bottom of the filter unit, where the screen is cleaned and collects the sludge.

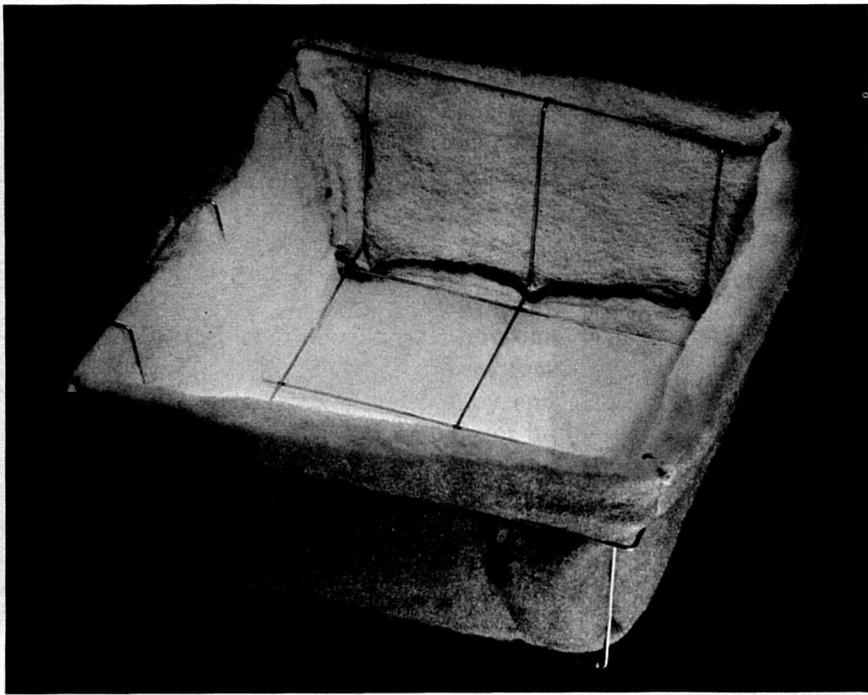


Figure 3. Medium-efficiency filters

pressed glass fibers or a good grade of paper fiber. The resistance to air flow is slightly higher than that of roughing filters and increases little when the filters are loaded with dust. Medium-efficiency filters must be discarded when they are loaded. These filters are generally used where freedom from rather large particles is desired and relatively clean air is required without a large reduction in flow rate. Medium-efficiency filters are used also as prefilters to reduce loading of higher efficiency filters. One type of prefilter, reported by Silverman and First (9), uses the unique principle of expanding the filter medium as the loading increases. This expansion extends the useful life of the filter to 2 or 3 times that of the fixed filters.

High-Efficiency Filters

High-efficiency biological filters (fig. 4) remove 90 to 99 percent of all particles in the 1- to 5-micron diameter range. The filter media are chiefly glass fibers, good grades of fiber paper, and asbestos fibers. The diameter of the fiber ranges from 1 to 5 microns. Resistance to air flow is higher than that of either the roughing or medium-efficiency filters, and increases appreciably as the filter loading increases. The air flow resistances of these filters may increase fourfold or more before discarding is necessary. Another characteristic of these filters is the ability of the medium to carry a heavy bacterial load before the resistance becomes excessive.

Ultra-High-Efficiency Filters

Filters that are classified as ultra-high-efficiency particulate filters (fig. 5) are used to achieve maximum removal of small biological and radioactive particles from air. Ultra-high-efficiency filters, for the purpose of this monograph, are classified as those that have an efficiency greater than 99.99 percent for removing bacterial particles having a diameter of 1 to 5 microns. Ultra-high-efficiency filters are used by the pharmaceutical, electronic, and other industries

to supply particle-free air to certain processes. These filters are more costly, have an initial flow resistance of 1 inch of water, and may be operated to resistances of 4 to 5 inches of water or more before replacement is necessary. Some of the materials used at present in these filters are cellulose-asbestos-fiber paper, glass and glass-asbestos fiber papers, ceramic fiber paper, compressed glass fibers, and composite beds of glass wool pads. Some of these materials are of recent development and are superior to the older materials because they do not support combustion. Ultra-high-efficiency filters have a higher resistance to air flow than the less efficient filters and must be discarded when they become loaded. Face air velocities of ultra-high-efficiency filter media range between 5 and 7 feet per minute. High air flow capacity in compact size is achieved by pleating the filter to provide increased surface area.

The ultra-high-efficiency filter was originally developed by the U. S. Army Chemical Corps for use in gas masks and in building filtration systems for removing bacteria and other particles not removed by charcoal filters. The original ultra-high-efficiency filter, unfortunately not fire proof, contained Bolivian or African Blue asbestos, esparto grass, and kraft fibers. It was known as Chemical Corps Type 6 medium. Emphasis by the Atomic Energy Commission on fire resistance has resulted in recent technological improvements on ultra-high-efficiency filters. Commercially available ultra-high-efficiency filters may now be obtained for fire-resistive (250° F) operation on up to high-temperature (2300° F) operation. Fire-resistive designs are constructed with glass or glass-asbestos fiber paper or ceramic fiber media. One of the glass media for the fire-resistive type is made into filter paper without a binder. Frames, separators, and cements are selected of materials that are incombustible, incorporate fire-suppressing chemicals, or have been impregnated to resist fire spread. In addition to these qualities, ultra-high-efficiency filters can be obtained with chemical and high-humidity resistance.

Although ultra-high-efficiency filters are excellent for removing all particles down to

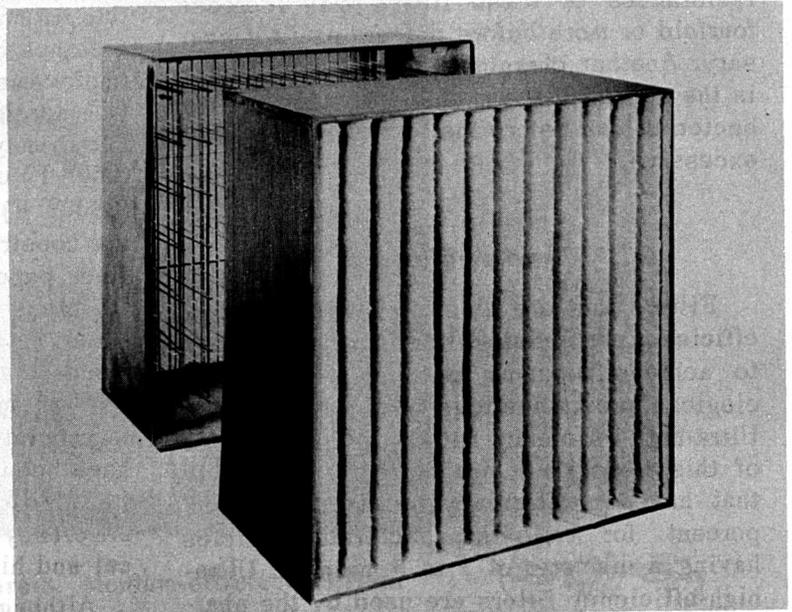
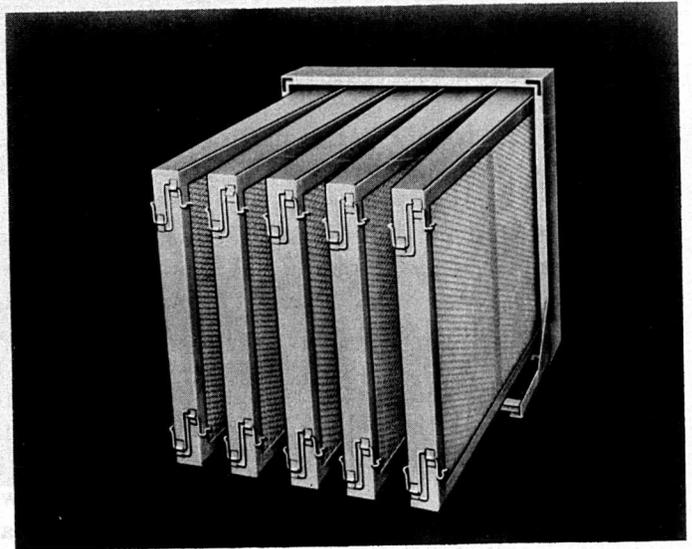
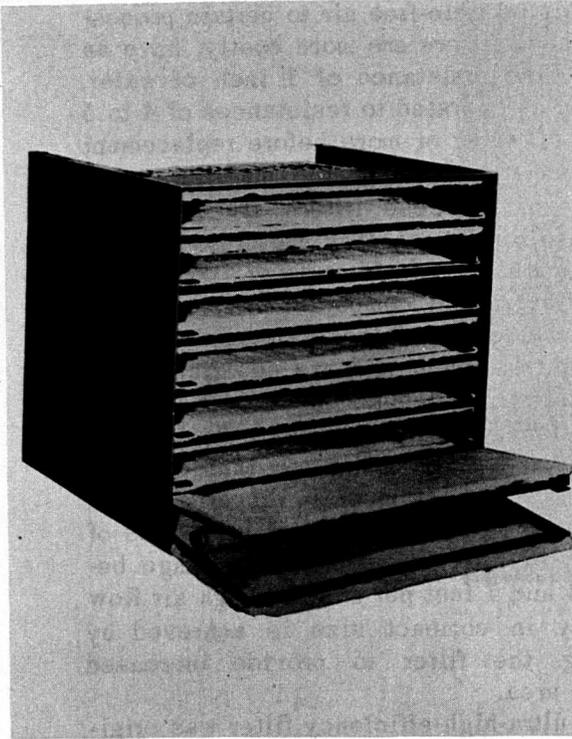


Figure 4. High-efficiency filters

at least 1 micron, it is certainly uneconomical to use them alone to remove large quantities of dust and other particles larger than 5 microns in diameter. The use of roughing, medium-efficiency, and possibly high-efficiency filters ahead of the more efficient and expensive ultra-high-efficiency type places the bulk of the loading on the less expensive filters, extends the life of the ultra-high-efficiency filters many-fold, and reduces total operating costs.

Air Washing

Air washing is another method for cleaning air. It is used chiefly for removing dust and other particles from air. Although it has been used in some instances for removing bacteria, its use for this purpose has not been developed extensively. Spray towers, zig-zag baffles, metal screens, and glass-fiber capillary cells represent some applications of this air cleaning principle. The most efficient air washers are those in which the suspended matter is impinged on a wet surface and then washed off. Only small amounts of particulate matter are removed by direct contact of the particles with liquid droplets. Washing alone is not a satisfactory method of removing bacteria from air, since the efficiency is usually relatively low. Air washers tested have been found to remove 20 to 80 percent of the bacteria in the 1 to 5 micron range (10). In some instances where the wash water is recirculated, the bacterial count of the air may actually increase because of reaerosolization of bacteria that have accumulated in the water. Air washers cannot be recommended for the removal of bacteria from air supplied to critical areas.

Air Scrubbing

Scrubbers are frequently used in chemical processes to bring a gas into close association with a liquid. Devices that might be classified as scrubbers are sometimes used in ventilating systems to control humidity. These scrubbers generally use a hygroscopic solution, which flows over numerous rows of multi-fin coils for temperature control of the

absorbent. In field evaluations by the authors, these units removed approximately 40 to 90 percent of particles 1 to 5 microns in diameter, with possibly higher efficiencies in the removal of larger particles (11). These scrubbers are an improvement over the washers using water, since there is no apparent reaerosolization of accumulated viable bacteria, and moisture does not accumulate on the heat transfer surfaces. The liquids used are generally bacteriostatic, if not bactericidal, and many of the viable organisms are killed when the liquid is heated to drive the water out of solution. In addition to the characteristics mentioned, scrubbers of this type operate automatically and require minimum maintenance. It is recommended, however, when these devices are used in ventilating systems supplying air to critical areas, that additional safeguards, such as high- or ultra-high-efficiency filters, be installed downstream.

Electrostatic Precipitation

Electrostatic precipitation is widely used for reducing air pollution caused by smoke and dust. It has been found satisfactory in many industrial air cleaning applications, removing over 90 percent of the particles. In this method, air passes through a high-voltage field where the suspended particles become charged and are then collected on electrodes of opposite charge.

The degree of particle removal depends on several factors, such as airflow velocity (which determines the time the particles remain in the field), voltage, the degree of plate loading, and the dielectric properties and size of the particles. Although electrostatic precipitators can remove a high percentage of bacteria and dust from air, they may not be as satisfactory as filters where a constant supply of clean air is required. In case of power failure, it would be possible for the contaminated air to pass through the devices. Such a condition could not be tolerated in many situations. Automatic closures could, of course, prevent this occurrence but would increase the cost of the installation. Some units also shut off

for a specific period to permit cleaning. During such periods, no particle removal is provided. Electrostatic precipitators that receive maximum maintenance have been shown in laboratory tests to remove or destroy approximately 90 percent of the microorganisms in the air (12). However, tests of some units under normal operating conditions have shown much lower efficiencies. In many installations, operating experience indicates that high humidity, small insects, or large particles in the entering air cause high-voltage arcing between the elements. Arcing due to large particles in the air can be reduced by the use of roughing or medium-efficiency filters ahead of the high-voltage section. Arcing due to high humidity can be corrected only by reducing the humidity of the air before it approaches the electrostatic elements. Arcing becomes so severe in some instances that the elements sustain permanent damage and the devices become inoperable.

Under optimum physical conditions and with satisfactory maintenance, electrostatic precipitators can be used in place of medium-efficiency filters. They should be equipped with high- or ultra-high-efficiency filters downstream if the air is to be supplied to critical areas. Without maximum maintenance, electrostatic precipitators may give a false sense of security.

Air Incineration

Although removal of bacteria by filtration is satisfactory in most situations, there

are some instances that require absolute certainty and dependability in the air purification process. For example, in research laboratories where infectious diseases are studied, high concentrations of pathogenic microbial aerosols may be created. Even though ultra-high-efficiency filters are quite efficient, if the concentration generated is extremely high (millions of organisms per cubic foot of air), passage of a few organisms will undoubtedly occur. Under such circumstances, air incineration is the method of choice. The organisms are not removed from the air, but instead are killed by heat.

Ultraviolet Air Sterilizers

Ultraviolet (UV) air sterilizers have been reported as being effective for inactivating organisms in an air stream (13). However, these sterilizers have the distinct disadvantage that the UV lamps must be cleaned and tested frequently. Furthermore, UV light has limited penetrating ability, and those organisms protected by dust may not be killed. Therefore, UV treatment of air is probably more useful against droplet nuclei and of less value against dust-borne organisms. Maintenance requirements and operational monitoring are even more severe and critical for UV installations than for electrostatic systems. It is recommended that considerable study be given to the availability of proper monitoring of UV energy output before UV lamps are installed for routine elimination of airborne bacteria.

III. Methods for Filter Evaluation

Nonbacterial Evaluation

Filters and filter materials are evaluated on the basis of their ability to remove particulate matter from an air stream. Some factors usually considered in testing a filter medium are: the flow resistance at various flow rates, the strength and diameter of fibers, the rate of clogging or breakdown

(temperature and/or resistance), and the minimum size of particle that the filter is capable of arresting. Methods for determining filter penetration by nonviable material are briefly described below, although they are not necessarily suitable for determining bacterial penetration through filters.

The National Bureau of Standards (NBS)

dust spot or blackness test (14) consists of challenging the test filter with a standard dust and drawing simultaneous samples of the unfiltered and the filtered air through filter papers. The air flow and filter areas are adjusted so that the spots on the filter paper are of equal blackness. The ratios of the areas of the black spots and air volumes sampled then indicate the effectiveness of the filter. Industrial filters are tested by injecting fly ash from a Cottrell precipitator into the air entering the filter to provide the challenging particles. Electrostatic precipitators and the more efficient types of fibrous filters are commonly tested by using atmospheric dust as a test material.

Methylene blue and other dyes have been used to test filters (15). In these tests, a solution of the dye is atomized into the influent air stream. Samples are collected on paper from each side of the filter so that the color density can be compared. The color density ratio indicates the degree of penetration.

A method developed by the American Society of Heating, Refrigeration, and Air Conditioning Engineers (ASHRAE) compares the weights of dust contained in equal sample volumes of influent and effluent air (16). This method can be used for testing any filter if the dust chosen is of the proper weight, particle size and shape. The Air Filter Institute (AFI) test procedure (17) is similar to that of the ASHRAE but is more sensitive because it specifies a standard test dust available from a commercial source.

Filter test methods employing smoke generated from different materials have been developed. The concentration of the smoke in the challenging and filtered air is determined by optical instruments, which measure the amount of light scattered by the smoke particles.

A method now used by many agencies for testing filters or filter media is known as the dioctyl-phthalate (DOP) test (18). Smoke is produced in a special generator by heating the dioctyl-phthalate and then mixing it with humidified air. When mixed with the proper amount of diluting air, this smoke contains particles of 0.3-micron diameter, at a concentration of approximately 40 grains per

cubic foot. The smoke is passed through the test filter at its rated velocity, and the concentration of DOP particles in the filtered and unfiltered air is measured by passing an air sample through a smoke penetrometer, a photoelectric instrument that measures the amount of light scattered by the passing DOP particles. A penetration of 0.001 percent can be measured by this method of testing. This test method is one of the least destructive from a loading aspect when evaluating an assembled filter unit.

Another method of testing filters uses triphenyl phosphate containing radioactive phosphorus. Particles removed by the filter or layers of filter media are assayed with a Geiger counter (19).

Bacterial Evaluation

All of the foregoing methods are used for evaluating filters or filter media designed to remove solid and liquid particles from an air stream. Although these methods may be used to simulate the challenging of a filter by viable bacterial particles, the true performance of a bacterial filter cannot be correctly determined without the actual use of viable bacteria suspended in air moving through the filter at rated capacity. Particles created from liquids or mineral solids will have different adhesive and electrostatic properties and may or may not remain on filter fibers as readily as bacterial particles. Also, some forces that cause deposition of particles may vary with the type of test material used. The bacterial test provides the most sensitive method of evaluating filters because it quantitates each individual organism that is collected in the sample. No standard procedure has yet been adopted to determine the efficiency of filters in removing biological organisms from an air stream. However, test methods used by the authors and described here have been found to be satisfactory, and are realistic since aerosols of viable organisms are used in the evaluation.

Several papers have been published on *Serratia indica* and *Bacillus subtilis* var. *niger* (*Bacillus globigii*) as organisms that may be used as test agents (20). The selec-

tion of the organism for evaluation is left to the discretion of the investigator. However, it has been found that the most consistent results are obtained when a heat-shocked suspension of *B. subtilis* var. *niger* spores is used. Spores are more resistant than vegetative bacteria, and can be collected with a filter type of bacterial air sampler, such as the cotton collector (21). If vegetative bacteria, such as *S. indica*, are used, a liquid impinger or agar-impaction type of air sampler should be used (22). The latter is the sampler of choice if naturally occurring microflora, in clumps or on dust particles, are being studied. The liquid-impinger type of sampler requires greater care in handling than does the cotton collector. Furthermore, since vegetative bacteria die off much more rapidly in the air stream than do spores, they do not give as reliable an index of filter arrestance. Methods for preparing and aerosolizing bacterial suspensions, and information on collecting and culturing media are given in Appendixes A, B, and C.

The system shown in figure 6 is a typical test arrangement that permits accurate deter-

mination of the bacterial arrestance of filters or filter media when spores are used as the test organism. The same system may be used with vegetative organisms for evaluating filters; however, a different type of sampler must be used, since cotton collectors are efficient only for the collection of spores. The system is relatively simple and can be set up fairly quickly. The bacterial organisms are nebulized into a chamber, where the cloud of bacteria is mixed with air. The aerosol is then drawn into the duct through the filter under evaluation at the rated face velocity, and is then exhausted through a blower to the outside. Aerosol samples are taken before and after the filter. If the test filter is not of the ultra-high-efficiency type, it may be advisable to place an ultra-high-efficiency filter in the blower discharge to prevent contaminating the atmosphere with the test bacteria.

The aerosol generator should have suitable capacity and characteristics so that an aerosol of proper concentration and particle size can be maintained. A discussion of this subject may be found in Appendix B.

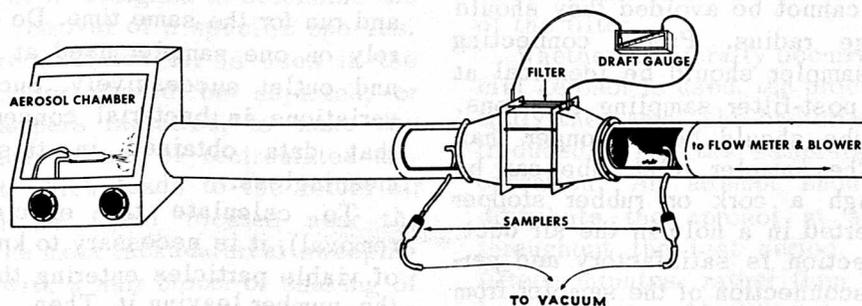


Figure 6. System for determining bacterial arrestance of filters using bacterial spores as a test organism

Field-Evaluation of Air Cleaning Systems

Although most air cleaning devices can be evaluated in the laboratory where conditions for such studies are favorable, in some instances it may be necessary to measure the cleaning efficiency with the device in place in the ventilation system. Such field-evaluation can be done by almost any bacteriology laboratory of moderate size with only a small expenditure for equipment.

If an entire system is to be evaluated, sampling points should be established at each end of the system. However, if only one component is under test, the system should be examined to determine whether a filter, washer, or other cleaning device is interposed between the sampler and the apparatus being evaluated. An attempt to measure the efficiency of an air washer behind a filter in a system probably would show the air approaching the washer to be low in bacterial count. Also, to test adequately a cleaning device, the device must be challenged by a relatively high bacterial concentration in the approaching air.

Isokinetic sampling of air before and after the filter may be desirable. To accomplish this, the air velocity in the duct should be determined with an anemometer. The size of the sampling probe should be such that the velocity of the air entering the probe is approximately the same as the velocity of the air in the air duct. The sampling probe should have a gentle curve with a radius of six inches or more. Hose connections should be as short and straight as possible, but where bends cannot be avoided they should have a large radius. Probe, connecting tubing, and sampler should be identical at the pre- and post-filter sampling locations. Probe and tube should be no longer than necessary. The sampler inlet tube can be passed through a cork or rubber stopper which is inserted in a hole in the air duct. Such a connection is satisfactory and permits rapid disconnection of the sampler from the probe.

Two points must be considered if accuracy is to be obtained: (a) the volume of

air sampled must be the same at both the inlet and outlet, and (b) care must be taken ~~to insure that both samplers are measuring~~ particles with similar characteristics. Methods for accomplishing the former have been detailed elsewhere (23). Error in the latter may occur when using samplers (such as the liquid impingement type), which reflect the total number of organisms rather than the total viable particles. If such samplers are used to evaluate air cleaning equipment that removes a greater portion of the larger particles and passes the small particles, an erroneous indication of efficiency may be obtained. Clumps of organisms collected upstream by these samplers will be broken up into individual organisms and, if efficiency is computed as described below, the efficiency of the equipment will appear higher than it actually is.

To avoid this, solid media devices including the slit, sieve, and Andersen samplers are recommended. The Andersen sampler lends itself well to the mechanics of the problem, and in addition to high total collection efficiency, it gives a rough guide to particle size. If the incoming air carries particles of nearly uniform size, a sampler may be used that is sensitive only to that particular particle size.

Before sampling is begun, allow several air changes through the air cleaner to assure uniform distribution of particles. Simultaneous samples should then be taken from the pre- and post-cleaner ports. When all preparations have been made, both pumps and samplers should be started simultaneously and run for the same time. Do not attempt to rely on one sampler used at both the inlet and outlet successively. Such large time-variations in bacterial concentration occur that data obtained in this manner are meaningless.

To calculate the efficiency (percent removal), it is necessary to know the number of viable particles entering the cleaner and the number leaving it. Then

$$\text{percent efficiency} = \frac{(\text{number in}) - (\text{number out})}{(\text{number in})} \times 100.$$

Tests With Naturally Occurring Microfloras

An air cleaning device may be tested under the conditions in which it normally operates, including such factors as species of organisms, particle size, concentration, temperature, and humidity. The results will be representative of the situation studied. However, conditions such as particle size may change, resulting in a change in cleaning efficiency from that indicated by the test. Within the limitations of the method, however, the evaluation of the cleaning efficiency of a ventilation system as installed can give very useful information.

Air systems using 100 percent fresh air usually present difficult evaluation problems because the incoming air may carry little contamination. In such situations, a sufficient number of replicates must be run to give total upstream and downstream colony counts, from all runs, of at least 1500. Total counts of this magnitude will give reasonable accuracy of results.

It should be realized, however, that when naturally occurring dust is used to challenge an air cleaner there is no control over size. In most instances the particles from this source will tend to be large. However, sufficient size distribution is usually obtained if several replicates are run.

If naturally occurring organisms are used, any good nonselective culture media may be used. Five percent blood agar with a heart infusion base is usually satisfactory. The bacteriological procedures are quantitative. No attempt is made to identify the organisms unless the test is designed to determine the efficiency of removal of a specific species.

If some recirculated air is used in the system, dampers should be adjusted, or temporary dampers installed, to make use of a maximum amount of recirculated air. Then a vent which leads to the return air duct should be found located near the floor. Even the most lackadaisical sweeping of the floor with a hair broom or shaking of dirty linen in front of this exhaust vent will simulate natural contamination and will produce sufficient bacterial contamination

in the air to evaluate the efficiency of the air cleaning equipment.

Tests With Artificial Microbial Aerosols

Although artificially produced aerosols of *Serratia marcescens* have been used to test some occupied buildings, this procedure is not recommended in occupied hospitals because debilitated patients should never be subjected to unnecessary contamination. However, coli bacteriophage, which at times has been given in human therapy, is safe. For unoccupied hospitals, *S. marcescens* is an excellent test organism. *B. subtilis* var. *niger* is more resistant, and therefore provides a more rigorous test of the facility, but the long persistence of the spore might be undesirable. Extensive experience with this test organism at Fort Detrick has shown that human inhalation of more than one million organisms is not harmful.

To establish the test aerosol, a nebulizer or aerosol generator should be placed in the air stream in front of the air cleaner at a distance sufficient to provide a uniform distribution of the aerosol across the face of the device. The recommended minimum distance is eight times the duct diameter. The air sampling probes should be placed in the direction of the air flow and as close as possible to the face of the filter. The location and number of samples taken should be representative of the total volume of air passing through the filter. It is recommended that, if possible, at least 0.1 percent of the total volume of air be sampled on each side of the filter.

Whether a naturally occurring or an artificial aerosol is used, the procedure is essentially the same. The bacterial aerosol is introduced. Then the samplers are placed in operation. An attempt should be made to distribute the aerosol at a uniform level throughout the test period, usually ten to fifteen minutes, rather than in one or more heavy bursts. At the end of the sampling period, remove the plates, incubate at the proper temperature, and count the colonies

when they have grown sufficiently. Calculate the removal efficiency by use of the formula given previously.

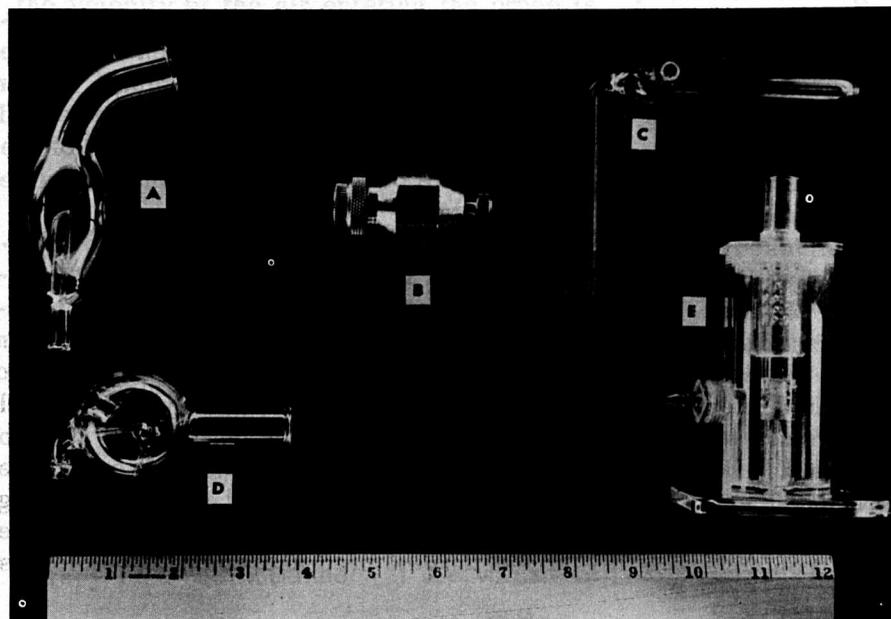
Viral Evaluation

Perhaps the most important characteristic of aerosols used in testing the efficiency of a filter is the particle size distribution. Considerable information is available on filter performance for the micron-sized particles such as bacteria (24), but little is known about the filtration of airborne viruses (25). Studies on particle deposition and filtration indicate that particles approximately 0.05 to 0.3 micron in diameter are the most difficult to remove from air. In general, viruses are not found in the air as naked, individual cells, but are attached to extraneous matter. However, for those organisms that may exist in the submicron sizes, information must be obtained.

All viruses are less than 0.3 micron in diameter and may be as small as 10 milli-

microns ($m\mu$). One of the chief reasons why there is no information on the efficiency of filters for virus aerosols is the lack of a suitable viable test aerosol. Problems associated with the development of a satisfactory test method include (a) the production of high-titered purified viral suspensions, (b) the development of an aerosol generator for the production of reproducible submicron uniparticulate aerosols, (c) the biological stabilization of the aerosol with variations in temperature and relative humidity, (d) the determination of the most efficient aerosol sampling methods, and (e) the measurement of the size distribution of the aerosol.

Logical candidates for a viral test aerosol are the bacteriophages. These are viruses which infect certain species of bacteria. The bacteriophages are not known to be infectious to man, and thus constitute a reasonably safe viral test agent. Bacteriophage suspensions used for the production of aerosols must be highly purified, otherwise the aerosol particles will



- A - DeVilbiss Nebulizer
- B - Binks Atomizer
- C - UCTL Atomizer
- D - Vaponefrin Nebulizer
- E - Dautrebande Nebulizer

Figure 7. Aerosol generators

consist mainly of the extraneous cellular materials present in the suspension, with the virus particles contributing little to the mass of the aerosol. Some techniques for virus purification include differential centrifugation, filtration, precipitation with acid, alcohol and salts, ion exchange chromatography, digestion with enzymes, and fractionation in liquid two-phase systems. Aerosol generators such as the Dautrebande, Vaponefrin, the DeVilbiss No. 40 nebulizers, and the University of Chicago Toxicity Laboratory (UCTL) atomizer (26), can be used for the production of uniparticulate aerosols with purified bacteriophage suspensions by

adjusting the viral concentration so that the aerosol droplets contain an average of one virus particle. These atomizers are shown in fig. 7.

Work is in progress at Fort Detrick on the development of a viral aerosol test method. Differential centrifugation has been used to purify and concentrate bacteriophage suspensions. Logical sequence of the work will be the determination of the aerosol particle size distribution, followed by studies on sampling and aerosol viability. The final phase of the investigation will be the use of the purified bacteriophage in air filtration and purification studies.

IV. Criteria for Filter Selection

Factors that should be considered in selecting a filter for a specific installation are: (a) degree of air cleaning desired; (b) toxicity or infectivity of airborne particles; (c) concentration and percentage of airborne particles; (d) volume of air to be cleaned per minute; (e) fire resistance; (f) mechanical strength; (g) time interval between filter cleaning or changes; and (h) cost of installation, operation, and maintenance. The architect, design engineer, or plant engineer must determine which of these factors are of primary importance to his particular situation. It may be advisable to measure particle size and dust loading to serve as a basis for estimate of filter life. As previously mentioned, the authors have categorized filters for removing bacteria into four groups: (a) roughing, (b) medium-efficiency, (c) high-efficiency, and (d) ultra-high-efficiency filters (figs. 2 through 5).

In general, the filter that removes the minimum necessary amount of contamination should be selected, since total cost is directly proportional to the filtration efficiency (27). When it is necessary to remove all bacteria from the air, as is desirable in certain hospital or laboratory sterile

areas, or industrial situations, one of the ultra-high-efficiency filters should be selected. Where considerable concentrations of pathogenic bacteria are present in exhaust air, such as that from bacteriological work hoods, an ultra-high-efficiency filter should be used. Under such circumstances, consideration should also be given to the use of air incineration.

In many instances where total removal of bacteria is not a requirement, a less costly air filtration system such as the high-efficiency filter may be satisfactory. Some of the less critical hospital areas or manufacturing processes require only medium-efficiency filters.

It has been shown that bacterial aerosols may be generated within a room by the presence of humans (28), or by the inanimate reservoirs of contamination. Building ventilation systems may remove this contamination by (a) streamlined flow across the entire cross section of the room, or (b) dilution of the room air by turbulent mixing with incoming air. Fully streamlined flow has not been achieved and present practice uses the latter method almost exclusively.

In a theoretical system using streamlined

flow, the contamination would be washed away from the source by clean air and moved to the exhaust at approximately the concentration level at which it was generated. Under these conditions, the cleaner the entering air, the cleaner would be the total room air.

With turbulent flow, it is immediately obvious that even if all air entering the room is sterile, this sterile air mixing with the room air merely dilutes and carries away a portion of any aerosol generated within the room and does not assure a sterile environment. If ventilation and generation of the aerosol within the room remain constant, the concentration of the organisms in the air will, in time, reach an equilibrium or steady concentration. The exact value of this concentration will depend, of course, upon the rates at which the aerosol is being produced, the methods and velocities by which the diluting air is supplied to the room, the degree of contamination, if any, of the incoming air, and the exhaust locations and velocities by which the mixture is removed.

Air Filtration Requirements for Hospitals

Critical Areas

The importance of efficient air purification systems for hospitals has been mentioned earlier. The contamination of concern in hospitals is usually produced in the room itself, rather than brought into the room from the outside. In most cases, outside air contains few viable organisms, and except in unusual situations only a small percentage of these are pathogens. The exception to this statement is the presence in many locations of the spores of *Clostridium perfringens* and possibly *Clostridium tetani*. Although the number of spores per cubic foot is low, the large volume of air moving through the air conditioning and ventilating system may cause the accumulation of a considerable number of organisms on the horizontal surfaces in the spaces served. This possibility should be reduced to a minimum by providing an air cleaning system of maximum efficiency to clean any air supplied to particularly sensitive areas such

as operating rooms and central supply services.

On the other hand, hospital personnel and patients have been shown to carry pathogenic organisms in their respiratory tracts and on their skin and clothing (29). The same organisms are all too frequently found on the floor and other surfaces of rooms where movement within the room can transfer them to the air. Staphylococci are frequently released into the air from humans (30). *E. coli* and tubercle bacilli are also released to the atmosphere from humans and the latter may be of considerable danger, as has been shown by the classic work of Riley, Wells, and others (31). Thus, there is reason to believe that potentially dangerous bacterial aerosols are being generated continuously by humans in the hospital environment. Surgical techniques of scrubbing, gowning, and the use of special types of face masks (32) that filter the organisms on both the inhalation and exhalation cycles will do much to reduce direct human contamination of the environment.

This raises the question as to whether it is necessary that every organism be removed from the recirculated air in the turbulent hospital ventilation systems found most commonly in the United States, or if one need only remove a sizeable fraction of these organisms. This problem is capable of mathematical analysis, and a simple model, containing what is hoped are practical parameters, has been developed.* The solution is in general terms, and the formula (Appendix D) can be used to make calculations for any type of situation, by substituting values for room size, ventilation rate, filter efficiency, etc. It is applicable for all situations where an aerosol is being generated within a closed space and is being continuously removed by filtration or by dilution with pure air.

The model assumes a situation that might occur in a typical hospital room. The following parameters are given:

Assume a room of approximately 5,000 cubic feet (20 by 20 by 12 feet), the air

*Prepared by Floyd H. Taylor, Fort Detrick, Maryland.

of which is clean at the start. Assume that there are ten air changes per hour and that the air is filtered during each change (table 2). For this model, 100 percent recirculation is assumed without the introduction of outside air. The filters are assumed to be 30, 60, 90, or 100 percent efficient. The latter efficiency is the same, of course, as bringing in completely clean air. Complete mixing of the air in the room is assumed.

No data are available on the concentration of organisms one would expect to be released by hospital personnel, either through their respiratory system or by dislodging organisms resting on surfaces. In an effort to approach reasonable parameters for these values, it may be assumed that 1000, 10,000 or 100,000 organisms per minute are being generated by the humans in the room. This range of values includes those that might be encountered in inhabited rooms, including hospital wards or operating rooms. Higher values would probably be encountered

only in areas where some special activity was carried out, such as the handling of soiled linen (33,34).

In the solution to the problem the value given to contamination rates has no effect on the choice of filters, because the equilibrium concentration reached is directly proportional to the contamination rate for any particular filter efficiency. It also is interesting to note that with these parameters the steady or equilibrium state is essentially reached within the first hour and is reached a little more rapidly with the more efficient filters. Table 2 shows that the concentration figure (equilibrium) for a 60 percent efficient filter is 2.00000, for a 90 percent filter 1.33333, and for a 100 percent filter 1.20000. There is considerable reduction in concentration of organisms between 60 and 90 percent efficient filters, but comparatively little reduction between the 90 and 100 percent filters. Thus, there is considerable benefit in using a relatively efficient (90 percent) filter as opposed to a relatively inefficient

TABLE 2. ROOM CONTAMINATION IN ORGANISMS PER CUBIC FOOT AT END OF ONE HOUR AND AT STEADY STATE

Filter Efficiency, %	Organisms being generated per minute		
	1,000	10,000	100,000
30	3.80085* (4.00000)*	38.00852 (40.00000)	380.08520 (400.00000)
60	1.99504 (2.00000)	19.95042 (20.00000)	199.50420 (200.00000)
90	1.33316 (1.33333)	13.33163 (13.33333)	133.31630 (133.33333)
100	1.19994 (1.20000)	11.99946 (12.00000)	119.99460 (120.00000)

Assumptions: 5,000 cubic feet in room; clean at start. Then air changes 10 times per hour through filters. Complete mixing obtained at all times.

* First figure in the body of the table gives concentration in organisms per cubic foot reached at end of one hour. The second figure, in parentheses, gives the equilibrium or steady state concentration. For development of the mathematical solution of this problem, see Appendix D.

(60 percent) filter, but very little additional benefit is gained by using a filter which is essentially perfect, or by supplying completely clean air from an outside source if distribution in the room results in turbulence.

In view of these conclusions, use of the high-efficiency filter, rather than the ultra-high-efficiency filter, is recommended for general use in hospitals where turbulent mixing is employed. However, where critical areas such as operating rooms are involved, and any other area where absolute sterility is mandatory, an ultra-high-efficiency filter in connection with minimum turbulence displacement distribution should be used to produce clean air. Ventilation systems designed to displace contaminated air with clean entering air, with minimal turbulence mixing, employ multiple ceiling inlets and low side wall or baseboard-type exhausts with low velocities in an attempt to produce streamlined flow. Thus, any point in the room is being washed by air from above, and the contamination of the air at any elevation will depend only on the contamination introduced into the air at or above that point. However, completely non-turbulent displacement is a difficult, if not impossible, condition to obtain, and relatively high-velocity streams of sterile air may have to be used in place of low-velocity displacement. The nearest practical approach to such distribution should be seriously considered for critical areas.

Non-Critical Areas

Roughing filters, with an efficiency ranging from 10 to 60 percent, usually are all that is required for most air cleaning where there is no need to remove all the bacteria, pollen, and dust. Roughing filters are also required as prefilters to prevent excessive loading of higher-efficiency filters. Bacterial

filters of low efficiency are recommended only where the sole need is to prevent the accumulation of dust and lint in rooms equipped with air conditioners and ventilators.

The dust-loading capacity of any filter depends upon the weight of dirt per unit area that the filter can carry before the resistance across the filter reaches the point where power consumption is excessive, due to increased demands on the air pumping system, or the volume of filtered air becomes too low. The time required for a filter to become loaded depends upon the concentration of particles in the air and the volume of air being cleaned. Maintenance costs will depend on the time between necessary filter changes or cleaning and the cost of replacement filters or labor for cleaning non-automatic filters.

Some commercial filters have been evaluated for various applications at government installations. Tables have been prepared placing filters evaluated in one of four categories (tables 3 through 6). Factors such as maximum operating temperature, fire resistance, moisture resistance, and (at times) chemical resistance must be considered in selecting a filter for a particular situation. Furthermore, if filters are to be used at high temperatures, it is recommended that tests be conducted prior to permanent installation to determine that they will not fail due to combustion. This precaution is recommended because some users have reported that filters have failed at significantly lower temperatures than anticipated. The filters evaluated and listed in this report constitute only a small number of the filters that are commercially available. There are other filters that perform as well as those listed, and inclusion of any particular filter does not represent endorsement by the United States Government or by the authors.

TABLE 3. ROUGHING FILTERS
Particle Retention* 10 to 60 Percent**

Nomenclature	Manufacturer	Media	Capacity cfm/ft ² of face A	Face velocity ft./min.	Press. drop In of H ₂ O	Max. oprn. temp.
AAF type HV 2	American Air Filter Corp. Louisville, Ky.	Adhesive-coated V-crimped wire screen mesh	250 to 430	300 to 500	0.04 to 0.10	110°F
AAF PL24 w/type G media	American Air Filter Corp.	Glass filament	up to 250	250	0.06	250°F
Drico puffglass	Drico Industrial Corp. Passaic, N. J.	Spun glass fiber	32 to 1,000	300	0.08 to 0.11	175°F
Far-Air HP-2	Farr Filter Co. Los Angeles, Calif.	Pleated cotton fabric	250 to 435	250 to 435	0.045 to 0.115	225°F
Farr 44-68	Farr Filter Co.	Crimped screen and wire mesh	250 to 435	250 to 435	0.040	275°F

*One to five microns.

**Inclusion of any particular filter in this table does not constitute endorsement by the United States Government or by the authors.

TABLE 4. MEDIUM-EFFICIENCY FILTERS

Particle Retention* 60 to 90 Percent**

Nomenclature	Manufacturer	Media	Capacity cfm/ft² of face A	Face velocity ft./min.	Press. drop In. of H₂O	Max. oprn. temp.
AAF deep bed Type 100 FG	American Air Filter Corp. Louisville, Ky.	Fiberglass	50 to 250	250	0.24	700°F
AAF PL 24 frame Type 25 FG	American Air Filter Corp.	Fiberglass	50 to 250	200	0.09	400°F
Aerosolve 45	Cambridge Filter Corp. Syracuse, N. Y.	Glass fibers	up to 500	250 to 500	0.16 to 0.25	400°F
Expandure	Flanders Filters Riverhead, N. Y.	Fiberglass	250	250	0.38	200°F
Type CA	Microtron Corp. Charlotte, N. C.	Polyester/acetate adhesive coated	200 to 250	200 to 250	0.08 to 0.13	350°F
U-LOK	Union Carbide Development Co. New York, N. Y.	Dynel fibers	200 to 500	300	0.10	180°F

*One to five microns.

**Inclusion of any particular filter in this table does not constitute endorsement by the United States Government or by the authors.

TABLE 5. HIGH-EFFICIENCY FILTERS

Particle Retention* 90 to 99 Percent**

Nomenclature	Manufacturer	Media	Capacity cfm/ft ² of face A	Face velocity ft./min.	Press. drop In. of H ₂ O	Max. oprn. temp.
Multi-Pak w/** 50 FG	American Air Filter Corp. Louisville, Ky.	Glass fiber	125 to 250	250	0.42	400°F
Deep bed w/ 50 FG	American Air Filter Corp.	Glass fiber	40 to 200	200	0.42	400°F
Microtain	Cambridge Filter Corp. Syracuse, N. Y.	Glass-asbestos pleated	50 to 250	Up to 250	0.4	220°F to 800°F
Aerosolve 85	Cambridge Filter Corp.	Glass fibers pleated	125 to 500	250 to 500	0.22 to 0.32	400°F
Aerosolve 95	Cambridge Filter Corp.	Glass fiber pleated	125 to 500	250 to 500	0.35 to 0.45	400°F
HP-100	Farr Filter Co. Los Angeles, Calif.	Glass fiber pleated	250	250	0.20	275°F
HP-200	Farr Filter Co.	Glass fiber	250	250	0.38	275°F

*One to five microns.

**Inclusion of any particular filter in this table does not constitute endorsement by the United States Government or by the authors.

***These filters made to accommodate double thickness of media.

TABLE 6. ULTRA-HIGH-EFFICIENCY FILTERS
Particle Retention* More than 99.99 Percent**

Nomenclature	Manufacturer	Media	Capacity cfm/ft ² of face A	Face velocity ft./min.	Max. drop In. of H ₂ O	Max. opm. temp.
AAF Type F (glass)	American Air Filter Corp. Louisville, Ky.	Glass fiber and kraft paper or alum sep.	30 to 400	68 to 325	1.0	250°F to 1000°F
AAF Type F (ceramic)	American Air Filter Corp.	Ceramic asbestos fiber and alum sep.	30 to 250	250	1.0	1600°F to 2300°F
Cambridge Absolute	Cambridge Filter Corp. Syracuse, N. Y.	Glass fiber asbestos paper sep.	30 to 345	Up to 275	1.0	800°F
Magnamedia	Farr Filter Co. Los Angeles, Calif.	Glass fiber	30 to 400	Up to 250	1.0	Up to 1000°F
Airpure absolute glass F 600	Flanders Filters Riverhead, N. Y.	Glass fiber (F600)	30 to 400	Up to 320	1.0	850°F
Airpure absolute ceramic- asbestos	Flanders Filters	Ceramic-asbestos	50 to 250	Up to 250	1.0	1600°F
Ultra-Aire	Mine Safety App. Co. Pittsburgh, Pa.	Glass fiber	35 to 250	Up to 250	0.9	500°F

NOTE: 1. Capacities are in cfm per sq. ft. of face area, not total area of filter.

2. Face velocities are fpm for 1 sq. ft. of face area, not media velocity.

*One to five microns.

**Inclusion of any particular filter in this table does not constitute endorsement by the United States Government or by the authors.

V. Installation Suggestions

General Considerations

The installation of an efficient biological air cleaning system to serve certain areas of hospitals, research installations, industrial plants, or civil defense shelters does not in itself necessarily ensure freedom from biological contamination. To maintain the atmosphere of selected rooms at a low level of bacterial contamination, it is necessary to establish a system of differential pressurization within a building.

In a hospital, for example, clean air flowing into an operating room must be used for the purpose intended, namely to provide to the greatest extent possible a germ-free atmosphere for patients. Use of a pressurized air system minimizes interchange of air from areas such as corridors and work rooms where the concentration of bacteria will be higher than that normally found in operating rooms. The pressure differential between the operating rooms and the hallways adjoining them should be

from 0.1 to 0.3 inch of water, with the operating rooms having the higher pressure. To facilitate such a balance, the use of a cubicle or air lock as shown in fig. 8 is recommended. If one is entering or leaving the pressurized area through an air lock, the first door should be closed before the second door is opened. The use of mechanical or electrical interlocks to prevent doors being opened at the same time should be considered for doors that are frequently left open. Such an arrangement prevents a sudden drop of pressure and is the best safeguard against flow of contaminated air into clean areas. As an added precaution, it is good policy to provide a downward wash of clean air within the air lock from the ceiling through the floor to remove contaminated particles from the clothing of persons passing through the air lock.

Now let us assume that one is working in a bacteriological laboratory handling considerable quantities of pathogenic microorganisms. In such a situation, the laboratory

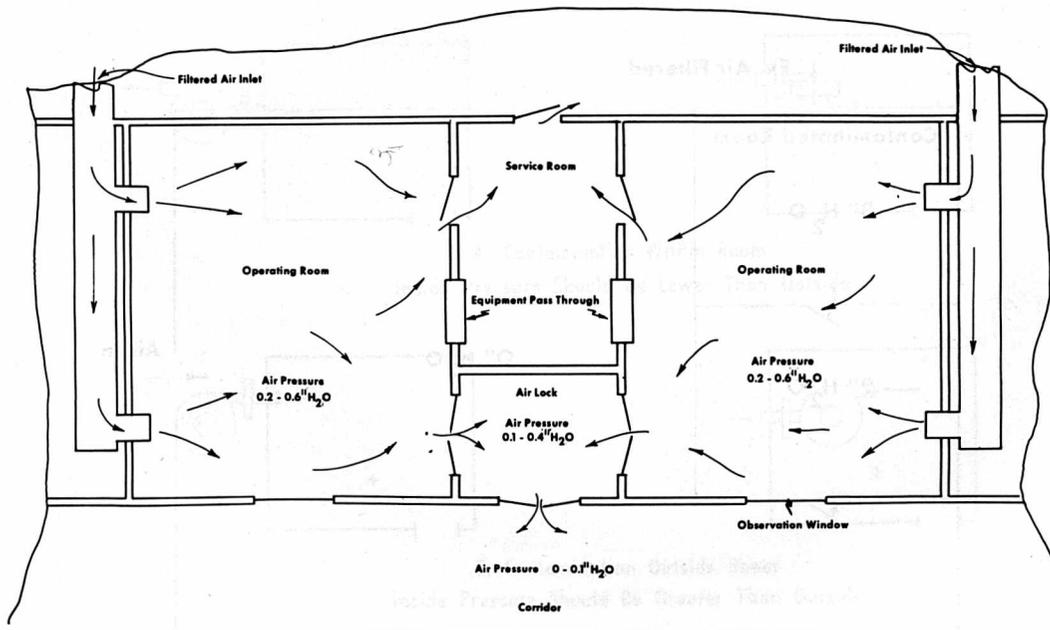


Figure 8. Air lock to reduce infiltration of contamination

must be under a reduced air pressure (35), and the laboratory air, which may contain pathogens, must be discharged through an efficient exhaust cleaning or incineration system and not permitted to enter hallways or areas where personnel can come in contact with the organisms (fig. 9). If there are several rooms in which pathogenic bacteria are handled, then graded degrees of reduced pressures should be provided, the more hazardous rooms having greater reduced pressure in relation to the less hazardous areas. In this manner the direction of air flow will always be toward the more dangerous room.

Air supplied to shelters, such as a civil defense shelter, should be filtered, however, contamination may enter through windows, cracks, or any small openings. Sealing of all unnecessary openings, where practical, is recommended. Contamination will be kept to a minimum if the inside air is maintained at a higher pressure than the outside air. Air required for normal ventilation may provide the necessary pressure (0.1 to 0.6 inch of water). If this is not sufficient, additional sealing of air locks or an increase in air supply will be required. This may be accomplished by using blowers of higher capacity

or additional blowers. In areas where it is critical that the air remain clean and free from contamination at all times, the need will justify the additional cost of pressurization.

If an air cleaning system is to operate efficiently, it must receive proper installation and maintenance. In order to achieve its maximum rated efficiency, a filter must be carefully installed in a properly prepared frame. The importance of a completely airtight installation becomes increasingly critical as higher initial operating pressure differentials are encountered. The likelihood of leaks increases significantly at these higher operating pressures. From a biological viewpoint, it should be noted that ANY LEAK, however small, is a potential source of trouble. Even the best designed filter system may be rendered ineffective by a small leak. The leakage of a few inanimate particles may not be significant, but the leakage of a few bacteria may result in serious consequences. Downstream beyond the filter, moist surfaces within the ventilation system may provide conditions suitable for the growth and reproduction of bacteria. Such areas represent potential contamination reservoirs. Subsequent aerosolization from

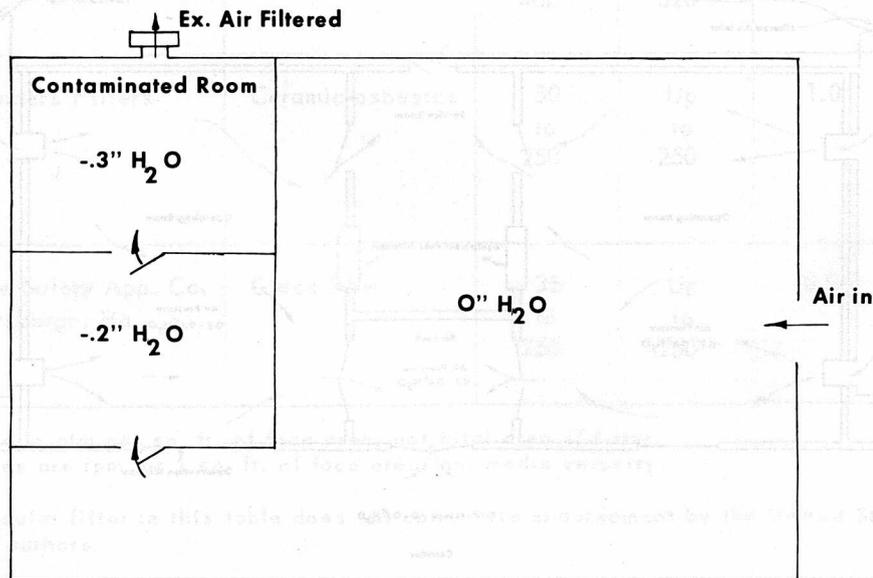


Figure 9. Pressurized system for bacteriological laboratory

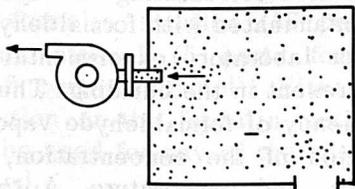
these contamination reservoirs may result in significant airborne bacteria emanating from the ventilation system. Any filter system which, when clean, imposes a pressure drop of 0.1 inch of water or more should be provided with air-tight gasketed seals.

Serious consideration should be given to multiple banks of filters in systems where clean air is required. A roughing filter upstream from the air handling and tempering equipment is recommended, while the higher efficiency filter should be located downstream. The choice of the latter filter will be determined by the quality of air desired. Ducts carrying contaminated air should be under negative pressure if possible; if under positive pressure, these ducts must be air-tight if they pass through a protected area.

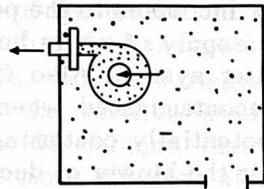
In designing a filter system, it is important that consideration be given to the relative position of the blower and filter as well as to the location of the system, *i.e.*, whether it is inside or outside the clean area. The proper method of installing the blower, to prevent the escape of unfiltered air in the event of leaks in the blower or duct system, is shown in fig. 10. When the air is forced through the filter, any blower leakage will be outward to the contaminated

air. When the air is drawn through the filter, leakage in the intake duct will be inward and must pass through the filter.

The installation of a pre-cleaner ahead of high-efficiency and ultra-high-efficiency filters is recommended. For this purpose, roughing filters, air scrubbers, or other types of air cleaning equipment can be used. Medium-efficiency filters as pre-filters are more expensive than roughing filters and, except for some automatic types, cannot be cleaned or rejuvenated and therefore must be replaced when they become loaded with dust and other contaminants. The use of a pre-cleaner increases the useful life of high-efficiency and ultra-high-efficiency filters many-fold, since it prevents loading of these filters by gross contamination. The addition of a pre-cleaner does not increase the power cost greatly, as air-flow resistance is in the order of 0.1 inch of water. Consideration also should be given to whether fire resistance of the filter unit is required. In the atomic energy program, fire-resistive construction of the filter unit is a chief requirement. Only under extenuating circumstances are cellulose-asbestos media used (*i.e.*, when hydrofluoric acid, which attacks glass, is present).



A. Contamination Within Room
Inside Pressure Should Be Lower Than Outside



B. Contamination Outside Room
Inside Pressure Should Be Greater Than Outside

FIGURE 10. RELATIVE POSITION OF FILTER AND BLOWER TO CONFINE CONTAMINATION INSIDE OR OUTSIDE ROOM

Several manufacturers of ultra-high-efficiency or high-efficiency filters recommend that filters be changed when the pressure exceeds 4 inches of water. Filter replacement is generally more economical than the cost of extra power needed to overcome the additional air-flow resistance of a dirty filter. A draft gauge should be installed in the filter system, with tubing leading to each filter, so that the pressure drop across each filter can be measured. The resistance of the filters should be checked regularly to detect loading before it becomes excessive.

A maximum allowable pressure drop and minimum flow is established for each type of filter, based on the capacity of the particular ventilation system.

In installations where a continuous supply of particulate-free air is necessary, such as civil defense shelters, an auxiliary filter system is desirable for use in case of primary unit failure or for use during maintenance of the primary filter. In such installations, an emergency power supply also should be available.

VI. Decontamination of Filters

Laboratory buildings in which work is carried out with pathogenic microorganisms should be equipped with bacteriological filters or air incinerators for purifying the air exhausted from the building. If filters are used, they must be changed when the resistance to air flow increases to the point where an insufficient supply of air is being drawn through the filter system. Also filters may have to be decontaminated when workmen must enter a potentially contaminated filter system to repair the blower or duct work, or to change a ruptured filter. On such occasions, filters must be decontaminated in place, or replaced by personnel wearing efficient respiratory protective devices to avoid inhaling pathogenic secondary aerosols created during replacement of the filters. Protective clothing should be worn to avoid possible contact of the body with pathogens and to avoid transfer of pathogens to clean areas. There are several methods by which filter systems can be decontaminated. The choice of method depends upon the system and facilities available. A filter unit or complete system may be decontaminated by chemicals or by heat.

Methods of Decontamination

Formaldehyde

Formaldehyde can be used to sterilize installed filters when air is exhausted to the outside. Recirculating air systems can be decontaminated with formaldehyde if personnel or laboratory experimental animals are not present in the building. The bactericidal efficiency of formaldehyde vapor is a direct function of the concentration, relative humidity, and temperature. A temperature of 75° F is desirable. The relative humidity should be above 70 percent since the effectiveness of the disinfectant decreases rapidly below this value. When a filter or filter system is decontaminated, the air flow should be reduced to a minimum so that a high vapor concentration can be maintained.

Most buildings are equipped with a heating system by which the temperature can be maintained at 75° F or higher. The relative humidity can be increased by spraying water from a vaporizer or from the same device used to disseminate the disinfectant. Even though the humidity has been raised, it is often necessary to continue to spray water

or inject steam at a reduced rate to maintain a high relative humidity. Steam ejectors, steam vaporizers, or other types of atomizers can be used, or a steam line equipped with a steam ejector can be permanently installed in the system.

Almost any method of disseminating formaldehyde into an air duct in suitable quantities is satisfactory when using this chemical as a filter decontaminant. For treating large systems, a large-capacity mechanical-type vaporizer can be used. Some of the disseminators that have been found satisfactory for vaporizing formaldehyde are shown in fig. 11 and listed in table 7.

Formaldehyde solution should be disseminated into the filter plenum at 1 ml. per minute for each cubic foot of air flow for 30 minutes; e.g., if the air flow is 600 cubic feet per minute, then 18,000 ml. of formaldehyde solution (600 x 1 x 30) will be disseminated in 30 minutes. When decontaminating an air duct, the outdoor wind direction is important. If the wind direction is from the air exhaust stack toward the air supply inlet then formaldehyde will be drawn into the building. This condition must be avoided.

Formaldehyde has the disadvantage of polymerizing on surfaces. The polymers are rather difficult to remove, but polymerization can be partially avoided by using a dilution of standard formalin solution (37 percent HCHO) with methanol (5 parts formalin solution to 3 parts methanol). Either the formalin solution or the formalin methanol mixture may be used for any of the applications described.

After decontamination, the filters are generally allowed to aerate overnight, after which they may be removed and discarded with minimum precautions.

Ethylene Oxide

Ethylene oxide is a satisfactory decontaminant, however its use is limited to gas-tight enclosures because the chemical is highly penetrating. To eliminate the hazard of the flammability of ethylene oxide, it is mixed with chlorofluorohydrocarbons or carbon dioxide. The filter system must be

divided by guillotine dampers so that the gas may be admitted to an airtight area. This type of a construction may be costly.

If the procedures previously discussed are impractical, as a last resort the filter unit can be removed from the ventilation system and placed in a modified autoclave, modified drum, or polyethylene bag to which a mixture of ethylene oxide and carbon dioxide or chlorofluorohydrocarbons (*i.e.*, freon) is admitted. If this latter method of decontamination is selected, personnel must be provided with adequate protection to avoid respiratory or body contact with viable pathogenic organisms that might be shaken off the filter while it is being removed from the ventilation system.

Self-Sterilizing Filters

Recent studies on the disinfection of hospitals have involved the use of air filters impregnated with a bactericide (36). Tests have been conducted on filters impregnated with an organic compound containing tin. A vegetative Gram-negative bacterium was used as the test organism. Results of these tests indicate that no appreciable reduction in passage of the viable microorganisms occurs when a filter is treated with this type of germicide. In these tests, it was found that the passage of viable organisms decreased in both treated and untreated filters as the relative humidity increased from 70 to 95 percent, thus both types of filters are more efficient at higher relative humidities. This phenomenon had been noted in previous tests with other types of filter systems. No commercial germicide that has been incorporated into surface materials or filters possesses any known significant ability to reduce the bacterial count on the filters unless it is surrounded by an environment of extremely high humidity.

Sterilization by Heat

Filters may be sterilized with heat by installing heating elements around noncombustible filters and sealing off the filter units so that the heat will be retained in a

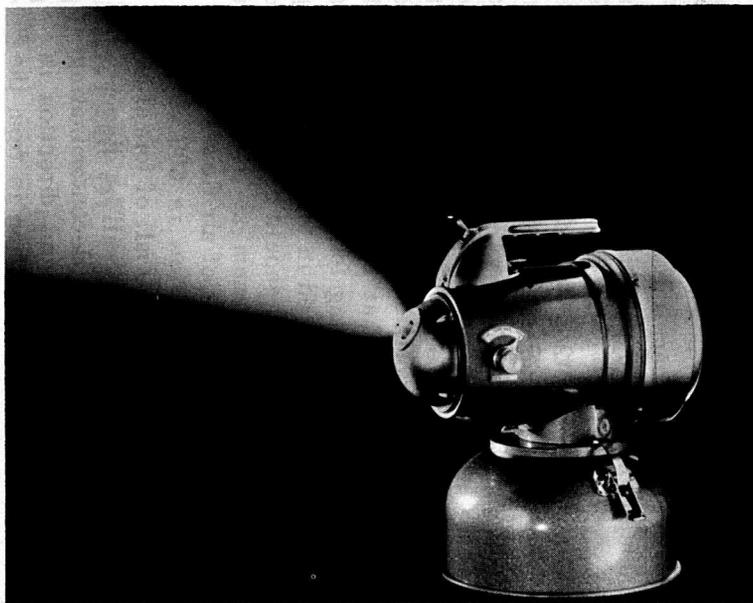
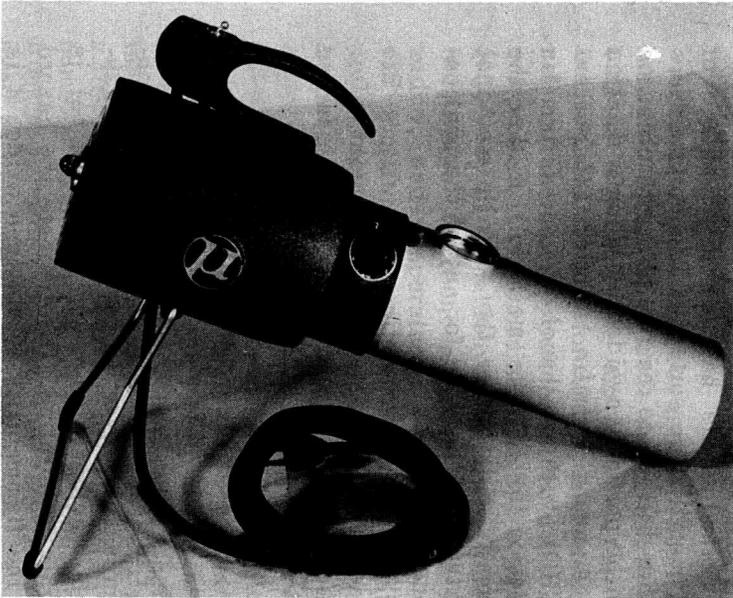
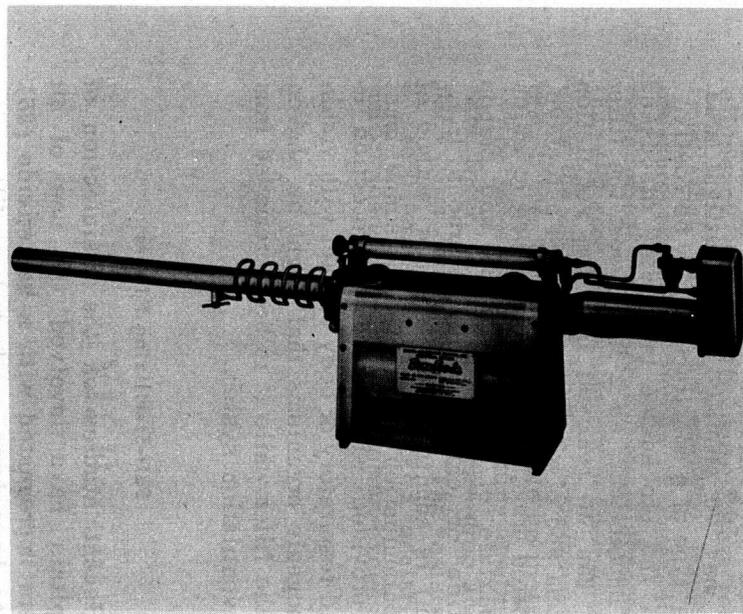


Figure 11. Disseminators for decontaminants

TABLE 7. COMMERCIAL SOURCES OF SOME SPRAYERS SUITABLE AS FORMALDEHYDE DISSEMINATORS*

Name	Container Capacity quarts	Manufacturer
Hydromist Vaporizer	1.4	Arnold Laboratories 7103 Laurel Canyon Blvd. North Hollywood, California
Dyna-Fog Sprayer	8	Curtis Automatic Devices, Inc. Westfield, Indiana
Microsol Mechanical Aerosol Generator	2	Silver Creek Precision Corp. Silver Creek, New York
Challenger Mechanical Sprayer	4	Z & W Manufacturing Corp. 30240 Cleveland Blvd. Wickliffe, Ohio

*Inclusion of sprayers in this table does not constitute endorsement by the United States Government or by the authors.

fireproof confined area. This method has been used in spun-glass air filter systems that exhaust air from bacteriological safety cabinets. If the filter cannot be decontaminated in place, it can be removed by masked personnel in protective clothing, placed in a bag, and incinerated, or it may be placed in a steam autoclave and decontaminated at 15 psi for 30 minutes.

Decontamination Clothing

Personnel who work with contaminated filters must be protected from infection by microorganisms in the filters. This protection includes wearing an efficient respiratory mask and protective clothing (fig. 12) or washable outer clothing such as laboratory overalls. The protective clothing should be

removed as soon as possible after potential contamination. It is always advisable to shower and don clean clothing as soon as possible after working in a potentially contaminated area.

Commercial sources of protective clothing include:

Mine Safety Appliances Company
201 North Braddock Avenue
Pittsburgh 8, Pennsylvania

Snyder Manufacturing Company, Inc.
1458 Fifth Street, N.W.
New Philadelphia, Ohio

Standard Safety Equipment Company
431 North Quenten Road
Palatine, Illinois

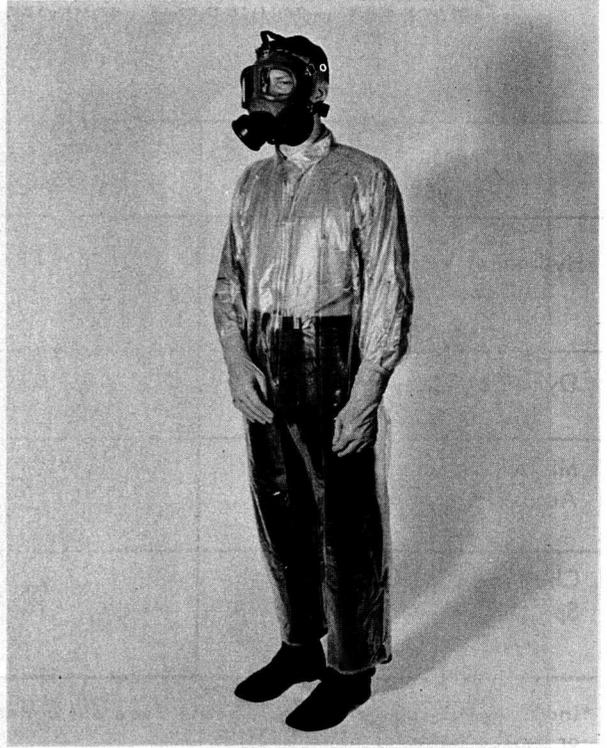
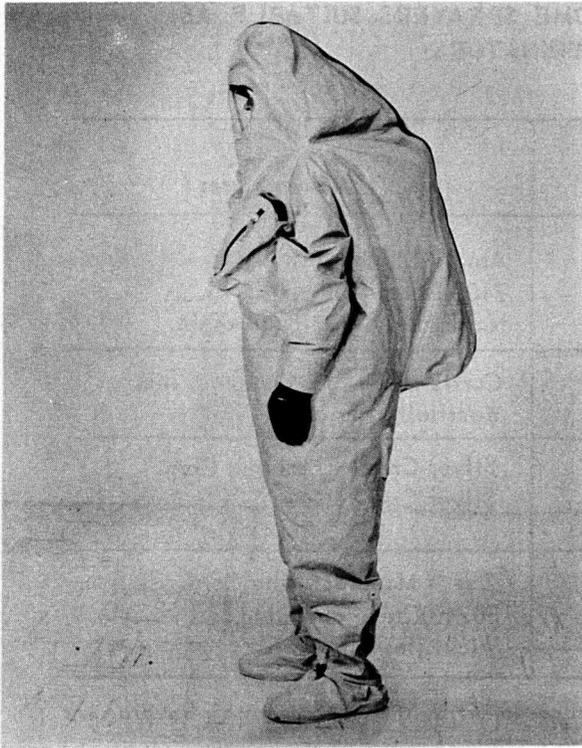


Figure 12. Protective clothing

Appendix A

Procedure for Growing *Bacillus subtilis* var. *niger* and *Serratia marcescens*

Stock cultures of *Bacillus subtilis* var. *niger* (*B. globigii*) and *Serratia marcescens* may be obtained from the American Type Culture Collection, 2112 M Street, N.W., Washington 7, D.C. Since these bacteria are conventionally regarded as nonpathogenic for man, no special safety precautions are required when working with them. Aseptic laboratory techniques should be used to prevent contamination of the air and laboratory facilities with the test bacteria and to prevent contamination of the test cultures with other organisms. Respiratory protection, in the form of a gas mask or a commercial respirator, should be provided whenever exposure to concentrated aerosols of *B. subtilis* or *S. marcescens* is anticipated.

Bacillus subtilis var. *niger*

Four tryptose agar (table A-1) slants are inoculated from the stock culture and incubated at 34° to 37° C for 24 hours. Following incubation, a heavy, yellow-orange pigmented colony growth will be visible on the agar surface.

Seven 250-ml. Erlenmeyer flasks, each containing 50 ml. of tryptose broth (table A-1) are inoculated with a large loopful of colony growth from the agar slants. These flasks are then incubated on a shaker for eight hours at 34° to 37° C.

After 8 hours, a 20-ml. inoculum from the Erlenmeyer flasks is added to each of 16 three-liter Fernbach flasks, each containing 250 ml. of tryptose broth. The Fernbach flasks are then incubated on a shaker for 5 to 6 days at 34° to 37° C.

After incubation, the liquid culture is centrifuged until the supernatant is clear. The supernatant is decanted and the cells are resuspended in 100 ml. of sterile distilled water and recentrifuged. Repeat this washing and centrifuging procedure 3 times. The cells are then resuspended in sterile

distilled water and transferred to a sterile bottle or flask. The procedure will yield a clean cell suspension containing approximately 90 percent spores with a concentration of 1×10^9 to 1×10^{10} spores per milliliter.

The spore suspension is heat-shocked by immersing the bottle in a 60° to 65° C water bath for 30 minutes to eliminate all vegetative cells. The 30-minute immersion time begins when the temperature of the spore suspension reaches 60° C. After heat-shocking, the concentration of the spore suspension is determined by the standard pour-plate method, using tenfold serial dilutions plated in triplicate with tryptose agar to obtain countable plates (30 to 300 colonies per plate). When not in use, all spore suspensions should be stored in a refrigerator at 4° to 6° C. At this temperature, the spores will remain viable for years without an appreciable change in concentration.

Serratia marcescens

Inoculate 2 tryptose agar slants from the stock culture of *S. marcescens* and incubate at 30° C for 24 hours. After incubation, a heavy, red-pigmented colony growth will be visible on the agar surface.

Inoculate 4 250-ml. Erlenmeyer flasks, each containing 50 ml. of tryptose broth, with a large loopful of colony growth from the agar slants. Incubate these flasks on a shaker for 18 hours at 30° C.

Add a 10-ml. inoculum from the Erlenmeyer flasks to each of 16 three-liter Fernbach flasks, each containing 250 ml. of tryptose broth. Incubate the flasks on a shaker for 18 hours at 30° C.

After incubation, the liquid culture is centrifuged until the supernatant is clear. The supernatant is decanted and the cells are suspended in sterile tryptose saline (table A-1) and centrifuged. Repeat this washing and centrifuging procedure 3 times. Resuspend the cells in 100 ml. of sterile

tryptose saline and transfer to a sterile bottle or flask. This procedure will yield a clean cell suspension containing approximately 1×10^9 cells per milliliter.

The concentration of the cell suspension is determined by the spread-plate method, using tenfold serial dilutions plated in

triplicate on tryptose agar, to obtain countable plates (30 to 300 colonies per plate). All cell suspensions should be stored in a refrigerator at 4° to 6° C when not in use. At this temperature, the cell suspensions will remain suitable for use for approximately 30 days.

The stock culture of *B. subtilis* var. niger may be obtained from the American Type Culture Collection, 5215 N. River, N.W., Washington, D.C. The spore suspension is conventionally prepared by nonpathogenic means, no special precautions are required when working with them. Aspartic acid laboratory technique should be used to prevent contamination of the air and laboratory facilities with the spores and to prevent contamination of the test cultures with other microorganisms. Respiratory protection in the form of a respirator or a commercial respirator should be provided whenever exposure to concentrated suspensions of *B. subtilis* or *S. cerevisiae* is anticipated.

Bacillus subtilis var. niger

Four tryptose agar (Table A-1) slants are inoculated from the stock culture and incubated at 34° to 37° C for 24 hours. Following incubation, a heavy, yellow-orange pigmented colony growth will be visible on the agar surface.

Seven 250-ml. Erlenmeyer flasks containing 50 ml. of tryptose broth (Table A-1) are inoculated with a large loopful of colony growth from the agar slants. These flasks are then incubated on a shaker for 18 hours at 34° to 37° C.

After 8 hours, a 20-ml. inoculum from the Erlenmeyer flasks is added to each of three-liter Fernbach flasks, each containing 250 ml. of tryptose broth. The Fernbach flasks are then incubated on a shaker for 8 days at 34° to 37° C.

After incubation, the liquid culture is centrifuged until the supernatant is clear. The supernatant is decanted and the cells are resuspended in 100 ml. of sterile distilled water and recentrifuged. Repeat this washing and centrifuging procedure 3 times. The cells are then resuspended in 100 ml. of sterile

tryptose saline and transfer to a sterile bottle or flask. This procedure will yield a clean cell suspension containing approximately 1×10^9 cells per milliliter.

The spore suspension is prepared by immersing the bottles in a 60° to 65° C water bath for 30 minutes to eliminate all vegetative cells. The 30-minute immersion time begins when the temperature of the spore suspension reaches 45° C. After heat-shocking, the concentration of the suspension is determined by the standard four-plate method using tenfold serial dilutions plated in triplicate with duplicate agar to obtain countable plates (30 to 300 colonies per plate). Spores are used in all spore suspensions should be stored in a refrigerator at 4° to 6° C. At this temperature, spores will remain viable for years without an appreciable change in concentration.

tryptose saline and transfer to a sterile bottle or flask. This procedure will yield a clean cell suspension containing approximately 1×10^9 cells per milliliter.

The spore suspension is prepared by immersing the bottles in a 60° to 65° C water bath for 30 minutes to eliminate all vegetative cells. The 30-minute immersion time begins when the temperature of the spore suspension reaches 45° C. After heat-shocking, the concentration of the suspension is determined by the standard four-plate method using tenfold serial dilutions plated in triplicate with duplicate agar to obtain countable plates (30 to 300 colonies per plate). Spores are used in all spore suspensions should be stored in a refrigerator at 4° to 6° C. At this temperature, spores will remain viable for years without an appreciable change in concentration.

TABLE A-1. FORMULAS FOR CULTURE MEDIA**Tryptose agar**

Bacto-tryptose	2.0%
Dextrose	1.0%
Sodium chloride	0.5%
Agar	2.0%
Water	to 1 liter

- Add ingredients to water and heat to boiling to dissolve.
- Adjust pH to 7.2-7.4.
- Dispense into flasks and test tubes and sterilize by autoclaving for 15 minutes at 15 lbs. pressure (121°C). Final pH should be 7.0 to 7.2.

Tryptose broth

Bacto-tryptose	2.0%
Dextrose	1.0%
Sodium chloride	0.5%
Water	to 1 liter

- Add ingredients to water and heat gently to dissolve.
- Adjust pH to 7.2-7.4.
- Dispense into flasks and sterilize by autoclaving for 15 minutes at 15 lbs. pressure (121°C).

Tryptose saline

Tryptose	0.1%
Sodium chloride	0.5%
Water	to 1 liter

- Add ingredients to water and heat gently to dissolve.
- Adjust pH to 7.2-7.4.
- Dispense into flasks and sterilize by autoclaving for 15 minutes at 15 lbs. pressure (121°C). Final pH should be 7.0 to 7.2.

NOTE: The individual ingredients can be obtained from most suppliers of biological media.

Appendix B

Aerosol Generation

For accurate assessment of penetration of bacterial filters, a challenge aerosol of particles of constant concentration and uniform size must be maintained throughout the test. Ideally, the aerosol should contain bacterial particles which are the size of each individual organism. Filters are evaluated with an aerosol consisting of particles 1 to 5 microns in diameter. The concentration of the challenging aerosol should be maintained at a constant high level, especially when ultra-high-efficiency filters are being tested. If filtration efficiency anticipated is greater than 99.99 percent and only a small volume of the effluent air is collected as a sample, it will not be representative. If a challenge aerosol of very high concentration is used or a large volume of the effluent air is collected in the sample, it will be more representative. It is suggested that the challenge aerosol have a concentration of 1×10^4 to 1×10^6 organisms per liter of air.

The organism selected as a test aerosol should be nonpathogenic, relatively stable in air, easy to produce in high concentrations, and simple to culture and assay. Many tests are performed with a spore-forming organism (*B. subtilis* var. *niger*) because it has all these characteristics.

When testing small sections of filter media or low-capacity filters, small glass or plastic atomizers may be used to spray the suspensions of test organisms (fig. 7 of text and table B-1 of this Appendix). Ex-

amples of these are the University of Chicago Toxicity Laboratories (UCTL) atomizer, the Vaponefrin, and the DeVilbiss No. 40 models. Each of these atomizers will discharge approximately 0.2 ml. of suspension per minute with an air requirement of 5 to 6 liters per minute at 15 psi. When properly used, all of these produce aerosols containing a high percentage of particles approximately 1 micron in diameter. The final size of the particles depends upon the characteristics of the spraying device, the physical characteristics of the suspension, and the relative humidity of the environment.

The Binks Series 50 (fig. 7 of text) and other similar two-fluid spray nozzles can be used where greater volumes of challenge aerosols are needed. One Binks nozzle is capable of generating a bacterial aerosol to raise the concentration in 50,000 liters of air per minute to 1×10^5 organisms per liter.

If still larger volumes of challenging aerosols are required, the centrifugal or spinning disc types may be used, such as the Microsol Mechanical Aerosol Generator (fig. 11 of text). This type of generator can discharge as much as 100 ml. of suspension per minute; however, it is not as efficient as the smaller generators for producing a high percentage of small particles. All generators should be positioned an adequate distance upstream from the filter to allow liquid droplets to evaporate and reach equilibrium and to allow the larger particles of the challenge aerosol to settle out before a sample is collected.

TABLE B-1. AEROSOL GENERATORS *

Type	Description	Recommended Spray Rate ml/min	Manufacturer
Binks Series 50	Direct-spray peripheral air-jet atomizer	2-10	Binks Corporation 3114-40 Carroll Avenue Chicago, Ill.
Dautrebande	Indirect spray	0.1	J. H. Emerson Co. 22 Cottage Park Avenue Cambridge, Mass.
DeVilbiss Model 40	Indirect spray	0.2	DeVilbiss Co. Somerset, Pa.
Mechanical Aerosol Fog Generator Model 202	Centrifugal spray	50-100	Silver Creek Precision Corp. Silver Creek, N. Y.
UCTL	All-glass, direct-spray peripheral air-jet atomizer	0.2	No Commercial Source
Vaponefrin Nebulizer	Indirect-spray atomizer or nebulizer	0.2	Vaponefrin Co. Upper Darby, Pa.

*Inclusion of aerosol generators in this table does not constitute endorsement by the United States Government or by the authors.

Appendix C

Media for Collecting and Culturing Microorganisms

The use of proper collecting and culturing media is of fundamental importance because the determination of the viable microorganisms contained in the sample depends on the number of bacterial colonies developing either directly on the nutrient collecting medium or in subsequent plate cultures of a sample.

Many collecting and culturing media are available. The selection of a nutrient medium will depend primarily on the nutritional requirements of the organism or organisms under study. The collected microorganisms must remain viable without a change in concentration until samples are taken for culture. Two collecting fluids that may be used for vegetative organisms are tryptose

saline and buffered gelatin. These media also are used as diluting fluids to obtain suspensions suitable for plating. Buffered saline and buffered water are used only for the collection of spores and other resistant microbial forms. Two media that may be employed for culturing the liquid samples or the water containing organisms washed from the cotton are tryptose agar and enriched nutrient agar. Surface plating methods are used.

If pathogenic organisms (e.g., staphylococci) are being studied, a suitable medium is one that contains a blood agar base.

The media listed in table C-1, as well as others can be prepared either with the dehydrated products from suppliers of biological media or according to formulas in textbooks and manuals on microbiology.

TABLE C-1. FORMULAS FOR MEDIA

	Gm/Liter of Distilled Water
Impinger Fluid	
Tryptose Saline	
Bacto-tryptose	1
Sodium chloride	5
Adjust pH to 7.0 ± 0.2.	
Buffered Saline	
Sodium chloride	8.5
Disodium phosphate (anhydrous)	5.8
Potassium dihydrogen phosphate (anhydrous)	3.5
Adjust pH to 7.0 ± 0.2	
Gelatin Diluent	
Bacto-gelatin	2
Disodium phosphate (anhydrous)	4
Adjust pH to 7.0 ± 0.2	
Buffered Water (Stock)	
Potassium dihydrogen phosphate	34
Adjust pH to 7.2. Add 1 to 2 ml. of stock to 1 liter of distilled water.	

	Gm/Liter of Distilled Water
Enriched Nutrient Agar	
Bacto-beef extract	3
Bacto-peptone	10
Yeast extract	5
Dextrose	10
Agar	20
Adjust pH to 7.0 ± 0.2	
Tryptose Agar	
Bacto-tryptose	20
Dextrose	1
Sodium chloride	5
Agar	15
Adjust pH to 7.0 ± 0.2	
Blood Agar	
Beef heart, infusion from	500
Bacto-tryptose	10
Sodium chloride	5
Agar	15
Adjust pH to 7.0 ± 0.2 . Add 5 percent fresh defibrinated horse, rabbit, sheep, or beef blood.	

Appendix D

MATHEMATICAL MODEL ON HOSPITAL VENTILATION

Let

N = number of organisms/cu ft present at time t in minutes

V = volume of room in cubic feet

K = number of complete changes of room volume/hour

b = total number of organisms/minute entering because of human presence

a = efficiency of the filter.

Then,

$\frac{NKV}{60}(1-a)\Delta t$ = total number of organisms/cu ft entering during the interval Δt because of the inefficiency of the filter.

$\frac{1}{V} b \Delta t$ = total number of organisms/cu ft entering during interval Δt because of contamination from individuals.

$\frac{1}{V} \frac{NKV}{60} \Delta t$ = total number of organisms/cu ft leaving during interval Δt .

ΔN = (total number of organisms/cu ft entering) - (total number of organisms/cu ft leaving)

$$\Delta N = \frac{NK}{60}(1-a)\Delta t + \frac{b}{V}\Delta t - \frac{NK}{60}\Delta t$$

$$\frac{\Delta N}{\Delta t} = \frac{b}{V} - \frac{KNa}{60}$$

$$\frac{dN}{dt} = \frac{b}{V} - \frac{KaN}{60} = \frac{b}{V} \left\{ 1 - \frac{aKV N}{60b} \right\}$$

$$\frac{dN}{1 - \frac{aKV N}{60b}} = \frac{b}{V} dt$$

$$-\frac{60b}{aKV} \int \frac{1}{1 - \frac{aKV N}{60b}} = \int \frac{b}{V} dt$$

$$-\frac{60b}{aKV} \ln \left\{ 1 - \frac{aKV N}{60b} \right\} = \frac{b}{V} t + C \quad \text{If}$$

$$-\frac{60b}{aKV} \ln \left\{ 1 - \frac{aKV N}{60b} \right\} = \frac{b}{V} t \quad \text{then,}$$

$$-\frac{60}{aK} \ln \left\{ 1 - \frac{aKV N}{60b} \right\} = t$$

$$\ln \left\{ 1 - \frac{aKV N}{60b} \right\} = -\frac{aKt}{60}$$

$$\left\{ 1 - \frac{aKV N}{60b} \right\} = \exp \left\{ -\frac{aKt}{60} \right\}$$

$$\frac{60b}{aKV} \left[1 - \exp \left\{ -\frac{aKt}{60} \right\} \right] = N$$

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References

- (1) Hatch, T. F.: Distribution and disposition of inhaled particles in respiratory tract. *Bact.* (Rev.) 25: 237-240, September 1961.
- (2) La Mer, V. K., Drozin, V. G., Kruger, J., and Catson, S.: Filtration of monodisperse solid aerosols. U. S. Atomic Energy Commission, Report N.Y.Q. 4526, 1953.
- (3) Langmuir, G., and Blodgett, K. B.: Smokes and Filters. (Supplement to Section I) O.S.R.D. Report 3460, 1944.
- (4) Green, H. L., and Lane, W. R.: Particulate Clouds: Dusts, Smokes and Mists. E. & F. N. Spon. Ltd., London, 1957.
- (5) Rodebush, W. H., Langmuir, I., and La Mer, V. K.: Filtration of aerosols and the development of filter materials. O.S.R.D. Report 865, September 4, 1942.
- (6) Rodebush, W. H.: Filtration of aerosols. Handbook on Aerosols, Atomic Energy Commission, U. S. Government Printing Office, Washington, D. C., 1950, pp. 117-122.
- (7) Ramskill, E. A., and Anderson, W. L.: The inertial mechanism in the mechanical filtration of aerosols. *J. Colloid. Sci.* 6: 416-428, October 1951.
- (8) Dallavalle, J. M.: Micromeritics, The Technology of Fine Particles, Chapter 8, Diffusion of particles, Pitman Publishing Company, 1948. pp. 167-168.
- (9) Silverman, L., and First, M. W.: Edge and variable compression filters for aerosols. *Ind. Eng. Chem.* 44: 2777, November 1952.
- (10) Unpublished data, Tech. Dev. Lab., Technology Branch, CDC, PHS, DHEW, Savannah, Ga.
- (11) Ibid.
- (12) Decker, H. M., Geile, F. A., Moorman, H. E., and Glick, C. A.: Removal of bacteria and bacteriophage from the air by electrostatic precipitators and spun glass filter pads. *Heating, Piping and Air Conditioning.* 125-128, October 1951.
- (13) Harstad, J. B., Decker, H. M., and Wedum, A. G.: Use of ultraviolet irradiation in a room air conditioner for removal of bacteria. *Appl. Microbiol.* 2: 148-151, May 1954.
- (14) Dill, R. S.: A test method for air filters. *Trans. ASHVE*, 44: 379, 1938.
- (15) Quarterly Progress Report, Protective equipment evaluation program. Y-12 Plant Carbide and Carbon Chemicals Division, October 1, 1949 to December 31, 1949.
- (16) American Society of Heating and Ventilating Engineers: Standard code for testing and rating air cleaning devices used in general ventilation works. *Trans. ASHVE*, 39: 225, 1933.
- (17) Code for testing air cleaning devices used in general ventilation. American Air Filter Institute, Station E, Box 85, Louisville 8, Kentucky, 1956.
- (18) Gucker, F. T., Jr., Pickard, H. B., and O'Konski, C. T.: Methods of testing smoke filters. Handbook on Aerosols, Atomic Energy Commission, U. S. Government Printing Office, Washington, D. C., 1950, pp. 123-136.
- (19) Vonnegut, B.: Design of apparatus, MIT-E1R1, for filter testing using triphenyl phosphate smoke, July 18, 1942.
- (20) Decker, H. M., Harstad, J. B., Piper, F. J., and Wilson, M. E.: Filtration of microorganisms from air by glass fiber media. *Heating, Piping and Air Conditioning*, 155-158, May 1954.
- (21) Wolf, H. W., Skaliy, P., Hall, L. B., Harris, M. M., Decker, H. M., Buchanan, L. M., and Dahlgren, C. M.: Sampling microbiological aerosols. U. S. Public Health Service, U. S. Government Printing Office, Washington, D. C., April 1959. 49 pp.
- (22) Ibid.
- (23) Ibid.
- (24) See reference (20).
- (25) Sadoff, H. L., and Almlöf, J. W.: Testing of filters for phage removal. *Ind. Eng. Chem.* 48: 12: 2199-2203. December 1956.
- (26) Rosebury, T.: Experimental Air-Borne Infection. Williams & Wilkins Company, 1947, p. 81.
- (27) Friedlander, S. K., Silverman, L., Drinker, P., and First, M. W.: Handbook on Air Cleaning Particulate Removal, Harvard University, Dept. of Industrial Hygiene and Atomic Energy Commission, U. S. Government Printing Office, Washington, D. C., September 1952. 89 pp.
- (28) Blowers, R., and Wallace, K. R.: III. Ventilation of operating rooms - bacteriological investigation. *Amer. J. Public Health*, 50: 4: 484-490, April 1960.
- (29) Nahmias, A. J., Godwin, J. T., Updyke, E. L., and Hopkins, W. A.: Postsurgical staphylococcal infections. *J. Amer. Med. Assoc.* 174: 10: 1269-1275, November 5, 1960.

- (30) Wolf, H., Harris, M. M., and Dyer, W. R.: *Staphylococcus aureus* in air of an operating room. J. Amer. Med. Assoc. 169: 1983-1987, April 25, 1959.
- (31) Riley, R. L., Wells, W. F., Mills, C. C., Nyka, W., and McLean, R. L.: Air hygiene in tuberculosis; quantitative studies of infection and control in pilot ward. Amer. Rev. Tuberc. and Pulmonary Disease, 75: 420-431, March 1957.
- (32) Adams, R., Fahlman, B., Dube, E. W., Dube, F. J. C., and Read, S.: Control of infections within hospitals, J. Amer. Med. Assoc. 169: 1557-1567, April 1959.
- (33) Duguid, J. P., and Wallace, A. T.: Air infection with dust liberated from clothing. Lancet, 2: 845-849, November 1948.
- (34) Bourdillon, R. B., and Colebrook, L.: Air hygiene in dressing room for burns and major wounds. Lancet, 1: 601-604, April 1946.
- (35) Wedum, A. G., Hanel, E., Phillips, G. B., and Miller, O. T.: Laboratory design for study of infectious disease. Amer. J. Public Health, 46: 9: 1102-1113, September 1956.
- (36) Hudson, P. B., Sanger, G., and Sproul, E. E.: Effective system of bactericidal conditioning for hospitals. J. Amer. Med. Assoc. 169: 1549-1556, April 1959.

